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# MICROPROPAGATION OF ORTHOSIPHON ARISTATUS THROUGH INDIRECT AND DIRECT ORGANOGENESIS

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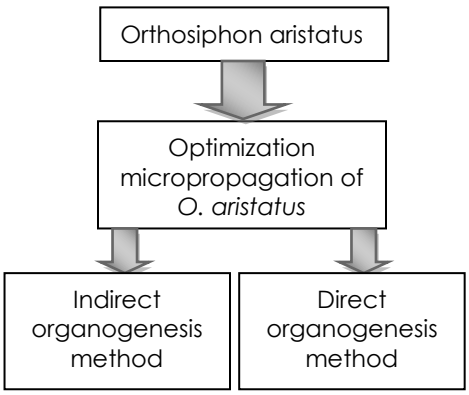
**Graphical abstract**




## Abstract

*Orthosiphon aristatus* is a herbal and ornamental herb from Lamiaceae family that has long been used as a diuretic for alleviating uric acid disease, gallbladder and kidney stone. Conventional propagation of this plant by stem cutting method faces major challenges notably due to the defect in roots formation, while the demand for this plant is high. This research aimed to establish a protocol for optimal micropropagation of *O. aristatus* through indirect and direct organogenesis method. Calli induction on MS agar medium supplemented with 2,4-D 0.5 mg $L^{-1}$ and kinetin 0.3 mg $L^{-1}$ resulted in 4.32 ± 1.49 g fresh weight of calli. Regeneration of shoots was successfully achieved on MS with 2 mg $L^{-1}$ BAP with 47 shoots from a single clump of calli. MS0 medium was found to be the best in inducing roots formation. Meanwhile, multiplication through direct organogenesis was optimally performed on MS supplemented with 2 mg $L^{-1}$ BAP with 9.00 ± 4.42 new shoots produced in 8 weeks after cultured. In conclusion, optimal multiplication shoots of *O. aristatus* has been achieved through callus formation and direct organogenesis.

*Keywords*: *Orthosiphon aristatus*, callus, organogenesis, BAP, 2,4-D



## 1.0 INTRODUCTION

*Orthosiphon aristatus* (Blume) Miq. (Syn. *O. grandiflorus* Bold.)), also called Kidney Tea, Cat's whiskers and Java Tea plant [20], is a popular medicinal and ornamental herb in European and South East Asia countries [21]. This perennial plant belongs to Lamiaceae family and is characterized by the upright stem, which can reach 1.5 m tall, the white-purplish flower with a filament that looks like Cat's whiskers and bushy appearance [21].

Traditionally, *O. aristatus* is used for alleviating kidney and urinary bladder-related diseases due to its diuretic activity [3, 12, 26]. This plant contains a high amount of Orthosiphonin glycosides and potassium, which could dissolve uric acid, phosphate, and oxalate from the body, thus prevent the formation of stones. Therefore, this plant has long been used for treating gallbladder stones, uric acid [54], and kidney stone [36]. Also, *O. aristatus* is also commonly used for diabetes, rheumatism, hypertension [54], renal ischemia [32], and for lowering blood triglycerides level [49]. It has been discovered that this plant has antioxidant [57] and antimetastatic activities [1].

During the commercial production of *O. aristatus*, their flowers are usually removed to increase the amount of health-promoting bioactive compounds in the leaves. The low germinative ability of the seeds could prevent generative plant

reproduction [38]. Consequently, the vegetative propagation method, notably by stem cutting, is employed for commercial production of this plant [55]. Even this technique faces some significant obstacles, such as scanty root and its delayed morphogenesis [41]. Therefore, there is an urgent need for a more efficient method of propagation to meet the market demand of this plant.

*In vitro*, culture is a potential technology for plant propagation in a rapid, uniform, and disease-free manner. The success of seed procurement through *in vitro* cultures is dependent on various factors such as the explant species and the physiological condition of the parent tree. Regeneration system in tissue culture can be through the path of direct and indirect organogenesis.

Direct organogenesis is one of the plant tissue culture techniques characterized by the formation of new buds or plant's organs directly from the mother plant. In the case of *O. aristatus*, direct organogenesis involves the formation of shoots from axillary bud explants by breaking the apical dominance of the apical meristem [11]. Meanwhile, indirect organogenesis occurs when callus, i.e., a collection of undifferentiated or partially differentiated cells, is formed from explants. The callus could proliferate and differentiate into plant organs [44].

The MS medium (Murashige & Skoog) is the most used basal media to support plant growth *in vitro*. Plant Growth Regulators (PGRs) are very important for the plant's physiological regulations, such as growth, development, and organogenesis [15]. Optimal multiplication could be achieved by adding specific types and amounts of PGRs, notably auxins and cytokinins, into the basal media [30]. Cytokinins favor the morphogenesis of axillary buds and the proliferation of shoot. The higher concentration of cytokinin to auxin ratio is required for the direction of induction of shoots on explants, and higher auxin to cytokinin ratio is necessary for callus initiation and proliferation of somatic cells. Among the synthetic cytokinins are BAP (6-Benzylaminopurine) and kinetin, while auxins are NAA (1-Naphthaleneacetic acid) and 2,4-D (2,4-Dichlorophenoxyacetic acid) [15].

Callus induction from leaf, petiole, and stem of *O. stamineus* has been reported by Lee & Chan [24] with the highest callus induction was observed in leaf explants. The usage of 2,4-D resulted in a high yield of callus induction of *Ipomoea obscura*, *Withania somnifera*, and *Cardiospermum halicacabum* [28]. Meanwhile, for direct organogenesis, positive outcome on shoot regeneration during organogenic segregation was found in media supplemented with cytokinin (BAP, TZD) in combination with auxin (IAA, NAA, IBA) [13, 53, 16, 42, 46]. Thus, this study was aimed to determine the effects of rapid propagation protocol of Indonesian *O. aristatus* by developing the optimization media for direct and indirect organogenesis under *in vitro* condition.

## 2.0 METHODOLOGY

### Explant Sterilization and Isolation

Axillary bud of *O. aristatus* was used as explant. The explant of *O. aristatus* was collected from Jakarta, Indonesia and maintained at Biotechnology Laboratory of Pelita Harapan University. The explants were washed with detergent and rinsed with running water to remove soil particles. The explants were dipped in 0.25 g of both bactericide and fungicide in 200 ml of water for 30 min, 2.25 mg $L^{-1}$ of antibiotic rifampicin for 4 hr, 2% of Clorox® bleach for 15 min, 1.5% of Clorox® bleach for 15 min, 1% of Clorox® bleach for 10 min, and 0.1% of $HgCl_2$ for 10 min. The explants were then rinsed twice with sterile water and planted into media in order to induce the growth of explants. Platelets were routinely propagated on Murashige and Skoog media supplemented with BAP 1 mg $L^{-1}$ and incubated at 18-20°C with flourescence lighting at the intensity of 2000 lux for 16 hr.

### Indirect Organogenesis: Induction, Proliferation, Regeneration of Calli

*In vitro* explant was obtained from *in vitro* established *O. aristatus* plantlets at Biotechnology Laboratory of Pelita Harapan University. Plantlets in healthy conditions were selected, and axillary buds were used as explants. The explants were cultured onto MS media supplemented with 0.5 mg $L^{-1}$ of 2,4-D and kinetin at the concentration of 0.1, 0.3, 0.5, 0.7, and 1 mg $L^{-1}$. Explant grown on MS0 media without any addition of PGRs served as a study control. The observation parameters were calli characteristics, number of shoots, and fresh weight of calli at the 10$^{th}$ week.

The induced calli were excised from explants, cut into several clumps with diameters around 0.5-1 cm, and each clump was grown on separate treatment media for calli proliferation, i.e., MS supplemented with 1 mg $L^{-1}$ of kinetin and 2,4-D at the concentrations of 0.5 mg $L^{-1}$, 1 mg $L^{-1}$, 1.5 mg $L^{-1}$, 2 mg $L^{-1}$, or 2.5 mg $L^{-1}$, or to treatment media for calli regeneration, i.e., BAP at the concentrations of 0.5 mg $L^{-1}$, 1 mg $L^{-1}$, 1.5 mg $L^{-1}$, 2 mg $L^{-1}$, or without any PGRs as a control. The observation parameters for calli proliferation experiment were calli characteristics, number of shoots, and calli fresh weight in the 10$^{th}$ week. Meanwhile, the observation parameters for the calli regeneration experiment were the number of shoots, plant heights, and a number of leaves.

Plantlets regenerated from embryogenic calli were subcultured to root induction media, i,e, MS supplemented with 0.5, 1, 1.5 mg $L^{-1}$, or without any PGRs (MS0 as a control). The observation parameter was the fresh weight of root the 8$^{th}$ week.

### Direct Organogenesis

The optimization of the multiplication plants, explants were grown on MS media supplemented with BAP 0, 0.5 mg $L^{-1}$, 1 mg $L^{-1}$, 1.5 mg $L^{-1}$, 2 mg $L^{-1}$, or 2.5 mg $L^{-1}$. Treatment of NAA at concentrations of 0, 0.5, 1, 1.5 , 2 mg $L^{-1}$. Explants were also grown on MS0 media without any PGRs as control experiments. The observation parameters were the number of shoots, plant height, number of leaves, and fresh weight of roots in the 8th week.

### Data Analysis

The experimental design of this study was a completely random design with 20 replications. The statistical analysis of the average number of shoots was done at the 8th week. The statistical analysis was performed using SPSS software. The mean value numbers were compared by ANOVA. ANOVA between multiple groups was performed using Duncan's method. The statistically significant was considered with p-value ≤ 0.05.

## 3.0 RESULTS AND DISCUSSION

### Induction, Proliferation, and Regeneration of Calli

In this research, calli could be induced from various parts of the plant, mainly from the leaves [24]. All treatment media were able to induce calli (Table 1). Characteristics of the induced calli were variable in color ranging from white, green to brown, globular, and compact (Figure 1a).

According to Aziz *et al*. [2], characteristics of embryogenic calli are white or pale yellow, compact, or often nodular and translucent. The compact cell will undergo a synchronized cell division. Calli of *O. aristatus* induced in this research was a combination of embryogenic and non-embryogenic calli, and the former will be followed with organogenesis to form a plantlet. No shoots were found on MS0 media, while the highest mean number of shoots (1.6 ± 0.87) and highest fresh weight of calli (5.53 ± 1.62 g) were observed on MS0 supplemented with 0.5 mg $L^{-1}$ of 2,4-D and the 1 mg $L^{-1}$ of kinetin. The plant growth regulator 2,4-D may define as a synthetic auxin that has strong activity.

**Table 1** Number of shoots and fresh weight of calli on induction media at 10th week

| Media | Number of shoots from calli | Fresh weight of calli (g) | Color of calli |
|---|---|---|---|
| T0 = MS + 0 mg $L^{-1}$ 2,4-D + 0 mg $L^{-1}$ kinetin | 0.00 ± 0.00$^{a}$ | 1.29 ± 0.38$^{a}$ | Brown |
| T1 = MS + 0.5 mg $L^{-1}$ 2,4-D + 0.1 mg $L^{-1}$ kinetin | 0.22 ± 4.28$^{a}$ | 2.69 ± 1.29$^{ab}$ | Light green |
| T2 = MS + 0.5 mg $L^{-1}$ 2,4-D + 0.3 mg $L^{-1}$ kinetin | 1.47 ± 0.85$^{b}$ | 4.32 ± 1.49$^{bc}$ | Greenish yellow |
| T3 = MS + 0.5 mg $L^{-1}$ 2,4-D + 0.5 mg $L^{-1}$ kinetin | 1.44 ± 0.83$^{b}$ | 5.16 ± 2.26$^{c}$ | Yellowish green |
| T4 = MS + 0.5 mg $L^{-1}$ 2,4-D + 0.7 mg $L^{-1}$ kinetin | 1.25 ± 0.55$^{b}$ | 4.06 ± 1.58$^{bc}$ | Brown |
| T5 = MS + 0.5 mg $L^{-1}$ 2,4-D + 1 mg $L^{-1}$ kinetin | 1.6 ± 0.87$^{b}$ | 5.53 ± 1.62$^{c}$ | Yellowish green |

*Note*. Each treatment consisted of 20 replications. Data were presented in the form of mean ± SD with the same letters indicate that there are no significant differences at p-value ≤ 0.05 according to Duncan analysis.

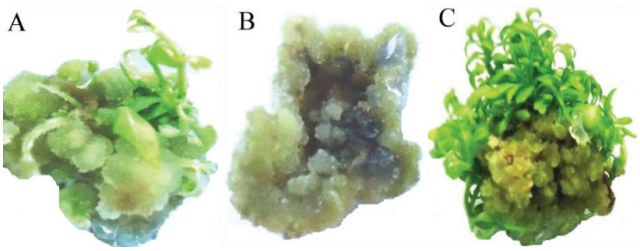



**Figure 1** Callus induction on MS media supplemented with 2,4-D and kinetin at 10th week. Note: A = induction of embryogenic callus and buds from single book stem explants, B = embryogenic callus proliferation as a source of adventitious shoots, C = adventive shoot proliferation

Statistically, the mean value numbers of shoots were obtained from treatment media supplemented with 0.5 mg $L^{-1}$ 2,4-D and 0.3, 0.5, 0.7, and 1 mg $L^{-1}$ Kinetin were significantly higher from MS0 and treatment media supplemented with 0.5 mg $L^{-1}$ 2,4-D and 1 mg $L^{-1}$ kinetin. On the other hand, the fresh weights of induced calli on media supplemented with 0.5 mg $L^{-1}$ 2,4-D along with 0.5 and 1 mg $L^{-1}$ kinetin were significantly higher compared to MS0 and treatment media supplemented with 0.5 mg $L^{-1}$ 2,4-D and 0.1 mg $L^{-1}$ kinetin. To stimulate cell division of somatic cells, the combination of 2,4-D and kinetin has a synergistic effect in the process of both differentiation and de-differentiation [44].

For calli induction, MS supplemented with 0.5 mg $L^{-1}$ 2,4-D and 0.3 mg $L^{-1}$ kinetin was found to be the optimum media for calli induction since its mean value number of shoots and fresh weight of calli were among the highest and not significantly different from the other media with a higher concentration of kinetin.

Plant growth regulator 2,4-D is a phenoxy synthetic auxin that plays an important role in promoting cell division [42], thus leading to callus induction and growth [7]. In accordance with the findings in this study, the other Lamiaceae plants, *Coleus vettiveroids* [22] and *Ocimum basilicum* [56],

also had their calli induced under treatment with 2,4-D.

The attempt to proliferate the induced calli under the treatment with 2,4-D and kinetin was failed to yield more embryogenic calli. The calli did proliferate, as indicated by the increasing fresh weight (Table 2, Figure 1b), but the color was brown to black, indicating dead cells. Therefore, it was better to subculture the calli directly from induction media to regeneration media.

**Table 2** Fresh weight calli on proliferation media at 10th week

| Media | Fresh weight of calli (g) | Color of calli |
|---|---|---|
| T0 = MS + 0 mg $L^{-1}$ 2,4-D + 0 mg $L^{-1}$ kinetin | 1.26 ± 0.39[a] | Black |
| T1 = MS + 0.5 mg $L^{-1}$ 2,4-D + 1 mg $L^{-1}$ kinetin | 2.09 ± 0.65[a] | Brown |
| T2 = MS + 1 mg $L^{-1}$ 2,4-D + 1 mg $L^{-1}$ kinetin | 3.99 ± 2.11[b] | Yellow |
| T3 = MS + 1.5 mg $L^{-1}$ 2,4-D + 1 mg $L^{-1}$ kinetin | 4.10 ± 1.89[b] | Yellowish green |
| T4 = MS + 2 mg $L^{-1}$ 2,4-D + 1 mg $L^{-1}$ kinetin | 2.79 ± 1.41[ab] | Brown |

*Note*. Each treatment consisted of 20 replications. Data were presented in the form of mean ± SD. with the same letters indicate that there are no significant differences at p-value ≤ 0.05 according to multiple range test Duncan's analysis.

In the present study, the highest percentage of calli regenerated was found on 1.5 mg $L^{-1}$ BAP (10%), followed by 2.5 mg $L^{-1}$ BAP (7.14%) (Figure 2). The highest number of shoots regenerated from a single clump of calli was achieved on MS media supplemented with 2 mg $L^{-1}$ BAP (47 and 23 shoots), followed by 2.5 mg $L^{-1}$ BAP (33 and 11 shoots). The regenerated shoots also had good viability, indicated by the production of many leaves, green in color, and not stunted (Figure 1c).

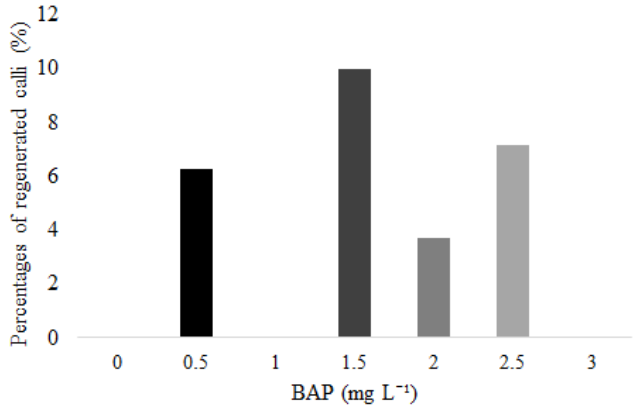



**Figure 2** The percentage of regenerated calli with different concentrations of BAP multiplication media

The embryogenic calli from induction media have to be subcultured to regeneration medium free of 2,4-D in order to increase the number of shoots. 2,4-D favors rapid cell division, while BAP could induce shoots direct or indirect organogenesis in *Stevia rebaudiana* [19] and *Lippia alba* [18]. Each embryogenic cell can be regenerated through somatic embryogenic which started from repeated cell divisions, globular, heart, and torpedo before finally becoming a plantlet [8].

Some calli showed green chlorophyll pigmentation at the beginning, but then it turned to black, which indicated a failure in shoot initiation. For calli regeneration, MS supplemented with 2 mg $L^{-1}$ or 2.5 mg $L^{-1}$ BAP was found to be the best since they could induce the regeneration of many shoots from a single clump of embryogenic calli.

The low percentage of regenerated calli may be due to several reasons 1) not all the cultured calli were embryogenic, thus can not produce shoots in spite of the optimal regeneration-inducing PGRs; 2) non-embryogenic calli could result from the imbalance between auxin and cytokinin in the plant. Dodds & Roberts [9] found that the level of PGRs in the medium must be balanced with the hormones in the primary explants, in addition to the endogenous hormones which were synthesized by the newly formed calli.

Therefore, the callus induction media have to be further optimized to yield more embryogenic calli that can regenerate into shoots; 3) the PGR used in the regeneration media is not optimal. The calli of some plants, such as *L. citriodora* [27], can not regenerate in media containing cytokinin only. The positive outcome of the combination of cytokinin and auxin for regeneration of the other plants has been observed by Bhagya *et al*. [5], Erisen *et al*. [13], Perez-Jimenez *et al*. [35], and Wadl *et al*. [53]; 4) The low regeneration capacity might be due to the long maintenance of calli on induction media [31, 23].

However, shoots from regeneration media can not directly be acclimatized since they did not produce enough roots and growth to overcome environmental stress [40]. Therefore, the individual shoot was subcultured to root induction media. In this research, root induction was achieved on all treatment media containing NAA (Table 3).

The fresh weight of roots on MS0 media was significantly higher than MS media supplemented with 0.5, 1, and 1.5 mg $L^{-1}$ NAA. The superiority of MS0 in inducing root formation seems deviated from some other studies which found the positive effects of NAA for root induction [40, 29, 39]. The NAA growth regulator is a synthetic auxin which has a strong activity power with a particular concentration that can inhibit root induction. The formation of buds, the natural biosynthesis of IAA transplanted in dried petals which have not applied NAA, shoots can form the roots.

**Table 3** Roots induction at 8th week

| Media | Fresh weight of roots (g) |
|---|---|
| T0 = MS + 0 mg $L^{-1}$ NAA | 0.17 ± 0.09$^{c}$ |
| T1 = MS + 0.5 mg $L^{-1}$ NAA | 0.01 ± 0.00$^{a}$ |
| T2 = MS + 1 mg $L^{-1}$ NAA | 0.01 ± 0.00$^{a}$ |
| T3 = MS + 1.5 mg $L^{-1}$ NAA | 0.09 ± 0.05$^{b}$ |

*Note*. Each treatment consisted of 20 replications. Data were presented in the form of mean ± SD with the same letters indicate that there are no significant differences at p-value ≤ 0.05 according to multiple range test Duncan's analysis.

However, this difference could be traced back to the different endogenous hormones each plant has. For example, *Hydrastis canadensis* also showed better root morphogenesis under treatment with a lower amount of cytokinin [4]. The roots induced on MS0 media in this study should be sufficient for further acclimatization. There are some ways to increase the number of roots, such as by using half-strength MS media [10], improving the number of shoot multiplication cycles, growing the explants in liquid media since it can provide a good aeration and support the development of root hairs, and added activated charcoal to the media [15].

## Direct Organogenesis

Besides indirect organogenesis through the formation of calli, multiplication of *O. aristatus* was also achieved by direct organogenesis method on MS media supplemented with five different concentrations of BAP (Table 4, Figure 3).

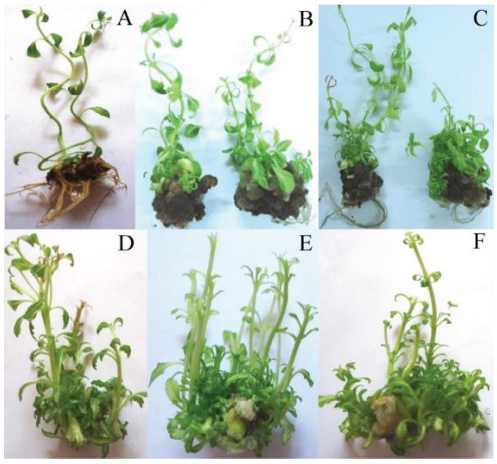



**Figure 3** Direct organogenesis on BAP multiplication media. Note: A = MS + 0 mg $L^{-1}$ BAP, B = MS + 0.5 mg $L^{-1}$ BAP, C = MS + 1 mg $L^{-1}$ BAP, D = MS + 1.5 mg $L^{-1}$ BAP, E = MS + 2 mg $L^{-1}$ BAP, F = MS + 2.5 mg $L^{-1}$ BAP

The highest mean value was recorded in a number of shoots, i.e., 10.33 ± 5.8 shoots per explant, it was achieved on media supplemented with 2.5 mg $L^{-1}$ of BAP, which is significantly higher than MSO, 0.5 mg $L^{-1}$ BAP, 1 mg $L^{-1}$ BAP, and 1.5 mg $L^{-1}$ BAP treatments. Treatment of 2 mg $L^{-1}$ BAP produced the highest mean number of leaves, i.e., 58.36 ± 18.92 leaves per explant, which was significantly higher than MS0 and not significantly different than the higher BAP concentration (2.5 mg $L^{-1}$ BAP). The mean value of plant height on MS0 media was significantly higher than 0.5 mg $L^{-1}$ BAP and 1 mg $L^{-1}$ BAP and was not substantially different from the other BAP treatment media. Multiplication of *O. aristatus* under treatment with NAA was also performed (Table 5). As expected, auxin doesn't favor shoot multiplication through axillary bud formation but rather enhance the apical dominance. Therefore, the mean value numbers of shoots on this NAA media were lower than on BAP media (Table 4), while the mean value of plant height on NAA was higher than on BAP media.

**Table 4** Multiplication through direct organogenesis on BAP with standard MS medium at 8th week

| Media | Number of shoots | Number of leaves | Plant's height |
|---|---|---|---|
| T0 = MS + 0 mg $L^{-1}$ BAP | 1.64 ± 0.74$^{a}$ | 28.43 ± 9.35$^{a}$ | 7.34 ± 2.20$^{c}$ |
| T1 = MS + 0.5 mg $L^{-1}$ BAP | 4.00 ± 1.07$^{ab}$ | 42.60 ± 13.44$^{ab}$ | 4.43 ± 1.81$^{ab}$ |
| T2 = MS + 1 mg $L^{-1}$ BAP | 6.50 ± 1.93$^{bc}$ | 49.20 ± 13.79$^{b}$ | 3.89 ± 1.74$^{a}$ |
| T3 = MS + 1.5 mg $L^{-1}$ BAP | 6.14 ± 3.48$^{bc}$ | 41.19 ± 19.94$^{ab}$ | 5.81 ± 1.75$^{bc}$ |
| T4 = MS + 2 mg $L^{-1}$ BAP | 9.00 ± 4.42$^{cd}$ | 58.36 ± 18.92$^{b}$ | 6.27 ± 1.32$^{c}$ |
| T5 = MS + 2.5 mg $L^{-1}$ BAP | 10.33 ± 5.83$^{d}$ | 57.13 ± 24.35$^{b}$ | 6.03 ± 1.88$^{bc}$ |

*Note*. Each treatment consisted of 20 replications. Data were presented in the form of mean ± SD with the same letters indicate that there are no significant differences at p-value ≤ 0.05 according to Duncan analysis.

**Table 5** Multiplication through direct organogenesis on NAA with standard MS medium at 8th week

| Media | Number of shoots | Number of leaves | Plant's height |
|---|---|---|---|
| T0 = MS + 0 mg $L^{-1}$ NAA | 1.64 ± 0.74$^{a}$ | 28.43 ± 9.35$^{c}$ | 7.34 ± 2.20$^{ab}$ |
| T1 = MS + 0.5 mg $L^{-1}$ NAA | 1.92 ± 1.31$^{a}$ | 11.67 ± 5.50$^{ab}$ | 5.25 ± 1.60$^{a}$ |
| T2 = MS + 1 mg $L^{-1}$ NAA | 1.36 ± 0.50$^{a}$ | 9.55 ± 4.34$^{a}$ | 8.95 ± 2.23$^{b}$ |
| T3 = MS + 1.5 mg $L^{-1}$ NAA | 1.73 ± 0.65$^{a}$ | 17.27 ± 6.92$^{b}$ | 9.41 ± 3.69$^{b}$ |
| T4 = MS + 2 mg $L^{-1}$ NAA | 1.31 ± 0.60$^{a}$ | 11.38 ± 4.98$^{ab}$ | 8.06 ± 3.35$^{ab}$ |

*Note*. Each treatment consisted of 20 replications. Data were presented in the form of mean ± SD with the same letters indicate that there are no significant differences at p-value ≤ 0.05 according to multiple range test Duncan's analysis.

Thus, multiplication of *O. aristatus* through direct organogenesis, MS media supplemented with 2 mg $L^{-1}$ of BAP was found to be the optimum one since it gave the highest number of shoots, leaves, and plant heights without being significantly different from the higher concentration of BAP. BAP has long been selected for the multiplication of various plants because of its ability to stimulate shoot proliferation [24, 50, 14, 47]. BAP was also found to be superior to kinetin as a cytokinin for *Gentiana kurro* [43], *Chlorophytum borivilianum* [37], and *Rotula aquatic* [25], *Typhonium flagelliforme* Lodd. [48]. The concentration of BAP applied in this research appeared to be optimal since it did not cause stunting, as commonly observed in the other plants [15, 6].

## 4.0 CONCLUSION

The optimum medium for *O. aristatus*'s embryogenic calli induction was MS supplemented with 0.5 mg $L^{-1}$ 2,4-D and 0.3 mg $L^{-1}$ kinetin, while for calli regeneration was MS supplemented with 2 mg $L^{-1}$ or 2.5 mg $L^{-1}$ BAP. The highest number of shoots regenerated from a single clump of calli was 47 shoots at 2 mg $L^{-1}$ BAP media. Maximum root induction was achieved on the MS0 medium. Shoots multiplication of *O. aristatus* through direct organogenesis method occurred at a high rate, i.e., forming 9.00 ± 4.42 shoots in 8 weeks after cultured on MS agar medium supplemented with 2 mg $L^{-1}$ BAP as the optimum media.

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## References

[1] Alsuede, F. S., Majid, A. S. A., Mohamed, B. K. A., Ismail, Z., Kadir, M. O., Majid, A. M. 2013. Antiangiogenic and Antimetastatic Activities of Cat's Whiskers Tea (*Orthosiphon stamineus*) extract against colon cancer Cell Line. *Cancer Research*. 73(3): B87.

[2] Aziz, S., Akeng, G., Kandasamy, K. I. 2000. Induction of Somatic Embryos from the Cotyledonary Tissue of Tongkat Ali (*Eurycoma longifolia*). *Journal of Tropical Medicinal Plants*. 1: 53-59.

[3] Beaux, D., Fleurentin, J., Mortier, F. 1999. Effect of Extracts of *Orthosiphon stamineus* Benth, *Hieracium pilosella* L., *Sambucus nigra* L. and *Arctostaphylos uva-ursi* (L.) Spreng in Rats. *Phytotherapy Research*. 13: 222-225.

[4] Bedir, E., Lata, H., Schaneberg, B., Khan, I. A., Moraes, R. M. 2003. Micropropagation of Hydrastis Canadensis: Goldenseal a North American Endangered Species. *Planta Medica*. 69: 86-88.

[5] Bhagya, N., Chandrashekar, K. R., Karun, A., Bhavyashree, U. 2013. Plantlet Regeneration through Indirect Shoot Organogenesis and Somatic Embryogenesis in *Justicia gendarussa* Burm. f., a Medicinal Plant. *Journal of Plant Biochemistry Biotechnology*. 22(4): 474-482.

[6] Bhojwani, S. S. & Razdan, M. K. 1996. *Plant Tissue Culture: Theory and Practice*. 1st ed. Amsterdam: Elsevier Science.

[7] Bonga, J. M. & von Aderkas, P. 1992. *In Vitro Culture of Trees* (Forestry sciences: v. 28). Netherlands: Springer Science.

[8] Butcher, D. N. & Ingram, D. S. 1976. *Plant Tissue Culture* (Studies in Biology, No. 65). London: Edward Arnold Limited.

[9] Dodds, J. H. & Roberts, L. W. 1982. *Back to the Roots: Experiments in Plant Tissue Culture*. New York: Cambridge University Press.

[10] Dubrovsky, J. G., Soukup, A., Napsucialy-Mendivil, S., Jeknic, Z., Ivanchenko, M. G. 2009. The Lateral Root Initiation Index: An Integrative Measure of Primordium Formation. *Annals of Botany (Lond.)*. 103: 807-817.

[11] Elangomathavan, R., Kalaivanan, P., Hariharan, P. and Beaulah, S. N. 2017. High Efficient Protocol for Callus Induction and Regeneration of a Medicinal Plant *Orthosiphon stamineus*. *International Journal of Advanced Research in Biological Sciences*. 4(1): 113-122.

[12] Englert, J. & Harnischfeger, G. 1992. Diuretic Action of Aqueous *Orthosiphon* Extract in Rats. *Planta Medica*. 58(3): 237-238.

[13] Erisen, S., Yorgancilar, M., Atalay, E. and Babaoglu, M. 2010. Prolific Shoot Regeneration of *Astragalus cariensis* Boiss. *Plant Cell, Tissue and Organ Culture*. 100: 229-233.

[14] Franca, S. C., Duarte, I. B., Moraes, R. M., Pereira, A. M. S. 1995. Micro-propagation of *Stryphnodendron polyphythum* (Barbatimao). *Plant Cell, Tissue and Organ Culture*. 42(3): 291-293.

[15] George, E. F. & Sherrington, P. D. 1984. *Plant Propagation by Tissue Culture-handbook and Directory of Comercial Laboratories*. Edington: Exegetics Limited.

[16] Ghimire, B. K., Seong, E. S., Goh, E. J., Kim, N. Y., Kang, W. H., Kim, E. H., Yu, C. Y., Chung, I. M. 2010. High-frequency Direct Shoot Regeneration from *Drymaria Cord* Willd. Leaves. *Plant Cell, Tissue and Organ Culture*. 100: 209-217.

[17] Gunawan, L. W. 1987. *Teknik Kultur Jaringan*. Bogor: Institut Pertanian Bogor Press. (Bahasa).

[18] Gupta, S. K., Khanjua, S. P. S., Kumar, S. 2000. *In Vitro* Micropropagation of *Lippia alba*. *Current Science*. 81: 206-208.

[19] Guruchandran, V. & Sasikumar, C. 2013. Organogenic Plant Regeneration via Callus Induction in *Stevia rebaudiana* Bert. *International Journal of Current Microbiology and Applied Sciences*. 2: 56-61.

[20] Indu Bala, J. & Ng, L. T. 1999. *Herbs: The Green Pharmacy of Malaysia*. Kuala Lumpur, Malaysia: Vinpress Sdn Bhd & the Malaysian Agricultural Research & Development Institute, Serdang, Selangor DE, Malaysia.

[21] Jaganath, I. B. & Ng, L. T. 2000. *Herbs: The Green Pharmacy of Malaysia*. Kuala Lumpur, Malaysia: Vinpress Sdn Bhd. In collaboration with the Malaysian Agricultural Research and Development (MARDI).

[22] Kamal, B. S., Padmaja, V., Dharmapalan, B. 2013. Induction of Callus from Leaf and Petiole of the Plant *Coleus Vettiveroids* K.C. *Scholars Academic Journal of Biosciences*. 1(6): 326-328.

[23] Krens, F. A., Menzel, T. R., Liu, C., Dees. D. C. T., Van Kronenburg, B. C. E. 2009. Oriental Lily Hybrids Engineered to Resist Aphid Attack. *Acta Horticulturae*. 836: 253-258.

[24] Lee, W. L. & Chan, L. K. 2004. Establishment of *Orthosiphon stamineus* Cell Suspension Culture for Cell Growth. *Plant Cell, Tissue and Organ Culture*. 78: 101-106.

[25] Martin, K. P. 2003. Rapid In Vitro Multiplication and Ex Vitro Rooting of Rotula aquatic Lour., a Rare

Rhoeophytic Woody Medicinal Plant. *Plant Cell Reports*. 21(5): 415-420.
[26] Matsubara, T., Bohgaki, T., Watarai, M., Suzuki, H., Ohashi, K., Shibuya, H. 1999. Antihypertensive Actions of Methylripariochromene a from *Orthosiphon aristatus*, an Indonesian Traditional Medicinal Plant. *Biological and Pharmaceutical Bulletin*. 22: 1083-1088.
[27] Mosavi, A. 2012. The Optimization of Lemon Verbena (*Lippia citriodora*) Medicinal Plant Tissue Culture. *International Journal of Agronomy and Plant Production*. 3(11): 561-565.
[28] Mungole, A., Awati, R., Dey, S., Chaturvedi, A., Zanwar, P. 2009. In Vitro Callus Induction and Shoot Regeneration in *Ipomoea obscura* (L.): Potent Indian Medicinal Plant. *Indian Journal of Science and Technology*. 2: 24-26.
[29] Munshi, M. K., Roy, P. K., Kabir, M. H., Ahmed, G. 2007. *In Vitro* Regeneration of Cabbage (*Brassica oleracea* L. var. capitata) through Hypocotyls and Cotyledon Culture. *Plant Tissue Culture and Biotechnology*. 2: 131-136.
[30] Mustafa, N. S. & Taha, R. 2012. Influence of Plant Growth Regulator and Subculturing on *In Vitro* Multiplication of Some Fig (*Ficus carcia*) Cultivars. *Journal of Applied Science Research*. 8(8): 4038-4044.
[31] Nakano, M., Sakakibara, T., Suzuki. S., Saito H. 2000. Decrease in the Regeneration Potential of Long-term Cell Suspension Cultures of *Lilium formosanum* Wallace and its Restoration by the Auxin Transport Inhibitor, 2,3,5-triiodobenzoic Acid. *Plant Science*. 158: 129-137.
[32] Nikolaev, S. M., Mondodoev, A. G., Skantanova, L. N., Glyzin, V. I., Azhunova, T. A. 1996. Pharmacotherapeutic Effectiveness of Nephrophyte in Cases of Renal Ischaemia. *Rastitel'nye-Resursy*. 32: 117-121.
[33] Ohashi, K., Bohgaki, T., Shibuya, H. 2000. Antihypertensive Substance in the Leaves of Kumis Kucing (*Orthosiphon aristatus*) in Java Island. *Yakugaku Zasshi*. 120: 474-82.
[34] Olah, N. K., Radu, L., Mogosan, C., Hanganu, D., Gocan, S. 2003. Phytochemical and Pharmacological Studies on *Orthosiphon stamineus* Benth. (Lamiaceae) Hydroalcoholic Extracts. *Journal of Pharmaceutical and Biomedical Analysis*. 33: 117-123.
[35] Perez-Jimenez, M., Carrillo-Navarro, A., Cos-Terrer, J. 2012. Regeneration of Peach (*Prunus persica* L. Batsch) Cultivars and *Prunus persica* × *Prunus dulcis* Rootstocks via Organogenesis. *Plant Cell Tissue Organ Culture*. 108: 55-62.
[36] Premgamone, A., Sriboonlue, P., Disatapornjaroen, W., Maskasem, S., Sinsupan, N. and Apinives, C. 2001. A Long-term Study on the Efficacy of a Herbal Plant, *Orthosiphon grandiflorus*, and Sodium Potassium Citrate in Renal Calculi Treatment. *Southeast Asian Journal of Tropical Medicine and Public Health*. 32(3): 654-660.
[37] Purohit, S. D., Dave, A., Kukda, G. 1994. Micropropagation of safed musli (*Chlorophytum borivilianum*), a Rare Medicinal Herb. *Plant Cell, Tissue and Organ Culture*. 39: 93-96.
[38] Rajendran, S. M., Chandrasekar, K., Sundersan, V. 2001. Ethanomedicinal Lore of Seithur hills-Southern Westen Ghats, Tamil Nadu. *Ethnobotany*. 13: 101-109.
[39] Ravanfar, S. A., Aziz, M. A., Kadir, M. A., Rashid, A. A., Sirchi, M. H. T. 2009. Plant Regeneration of *Brassica oleracea* subsp. italica (broccoli) cv Green Marvel as Affected by Plant Growth Regulators. *African Journal of Biotechnology*. 11: 2523-2528.
[40] Razdan, M. K. 1993. *An Introduction to Plant Tissue Culture*. New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.
[41] Reshi, N. S., Sudarshana, M. S., Rajashekar, N. 2013. Callus Induction and Plantlet Regeneration in *Orthosiphon aristatus* (Blume) miq. a Potent Medicinal Herb. *IOSR Journal of Pharmacy and Biological Sciences*. 6(3): 52-55.
[42] Sen, M. K., Nasrin, S., Rahman, S., Jamal, A. H. M. 2014. In Vitro Callus Induction and Plantlet Regeneration of Achyranthes aspera L., a High-value Medicinal Plant. *Asian Pacific Journal of Tropical Biomedicine*. 4: 40-46.
[43] Sharma, N., Chandel, K. P. S., Paul, A. 1993. *In vitro* Propagation of *Gentiana kurroo*-an Indigenous Threatened Plant of Medicinal Importance. *Plant Cell, Tissue and Organ Culture*. 34: 307-309.
[44] Sharp, W. R., Sondahl, M. R., Caldas, L. S., Maraffa, S. B. 1980. *Horticultural Reviews*. Conneticut, USA: AVI Publishing Co.
[45] Shen, X., Chen, J., Kane, M. E. 2007. Indirect Shoot Organogenesis from Leaves of Dieffenbachia cv. Camouflage. *Plant Cell Tissue Organ Culture*. 89: 83-90.
[46] Sianipar, N. F., Laurent, D., Purnamaningsih, R., Darwati, I. 2015. Genetic Variation of the First Generation of Rodent Tuber (*Typhonium flagelliforme* Lodd.) Mutants Based on RAPD Molecular Markers. *HAYATI Journal of Biosciences*. 22(2): 98-104.
[47] Sianipar, N. F., Verlina, Rosaria. 2016. Induction, multiplication and acclimatization of red betel plant (Piper crocatum Ruiz and Pav.) by in vitro organogenesis. *Jurnal Teknologi (Sciences & Engineering)*. 78: 35-40.
[48] Sianipar, N. F., Vidianty, M., Chelen, Abbas, B. S. 2017. Micropropagation of Rodent Tuber Plant (*Typhonium flagelliforme* Lodd.) from Medan by Organogenesis. *Pertanika Journal of Tropical Agricultural Science*. 40(4): 471-484.
[49] Sriplang, K., Adisakwattana, S., Rungsipipat, A., Yibchok-Anun, S. 2007. Effects of Orthosiphon Stamineus Aqueous Extract on Plasma Glucose Concentration and Lipid Profile in Normal and Streptozotocin-induced Diabetic Rats. *Journal of Ethnopharmacology*. 109: 510-514.
[50] Sudha, G. G. & Sooriamuthu, S. 1996. In vitro Propagation of Rauwolfia micrantha, a Rare Medicinal Plant. *Plant Cell, Tissue and Organ Culture*. 44(3): 243-248.
[51] Sumaryono, W., Proksch, P., Wray, V., Witte, L., Hartmann, T. 1991. Qualitative and Quantitative Analysis of the Phenolic Constituents from *Orthosiphon aristatus*. *Planta Medica*. 57: 176-180.
[52] Tezuka, Y., Stampoulis, P., Banskota, A. H., Awale, S., Tran, K. Q., Saiki, I., Kadota, S. 2000. Constituents of the Vietnamese Medicinal Plant Orthosiphon Stamineus. *Chemical and Pharmaceutical Bulletin*. 48: 1711-1719.
[53] Wadl, P. A., Dattilo, A. J., Vito, L. M., Trigiano, R. N. 2011. Shoot Organogenesis and Plant Regeneration in Pityopsis ruthii. *Plant Cell Tissue Organ Culture*. 106: 513-516.
[54] Wiart, C. 2002. *Orthosiphon stamineus Benth. Medicinal Plants of Southeast Asia*. Selangor: Prentice Hall.
[55] Wirjodarmodjo, H. I., Poernomo, S., Adiningrat, E. D. 1988. Application of Tissue Culture in Tropical Trees. *Biotrop Species*. 35: 97-101.
[56] Wongsen, W., Bodhipadma, K., Noichinda, S., Leung, D. W. M. 2015. Influence of Different 2,4-D Concentrations on Antioxidant Contents and Activities in Sweet Basil Leaf-derived Callus during Proliferation. *International Food Research Journal*. 22(2): 638-643.
[57] Yam, M.F., Basir, R., Asmawi, M.Z., Ismail, Z. 2007. Antioxidant and Hepatoprotective Effects of *Orthosiphon stamineus* Benth Standardized Extract. *American Journal of Chinese Medicine*. 35(1): 115-126.