In vitro regeneration of porang (*Amorphophallus muelleri* Blume) at several concentrations of BAP (benzyl amino purine)

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Abstract. There is problem about propagating porang by seed that caused it is not always available. Planting by bulbs, bulbil, or leaf cuttings need longer times caused by dormancy problems. Micropropagation by tissue culture is an alternative method that can be choose to fix the problem. This research is aim to obtain the best concentration of BAP to regenerate 'Madiun 1' variety of porang plants by shoots (organogenesis) formation. The research was carried out using complete randomized design with 7 treatments concentrations of BAP, that was: P1 (0 mg l^{-1} BAP), P2 (1 mg l^{-1} BAP), P3 (2 mg l^{-1} BAP), P4 (3 mg l^{-1} BAP), P5 (4 mg l^{-1} BAP), P6 (5 mg l^{-1} BAP), and P7 (6 mg l^{-1} BAP). Each treatment was consisted of 5 replications. Observation were made on number of budding, number of shoots, and shoot height. The data were analyzed using ANOVA, and the differences between treatments were tested using the LSD test at the 5% level. The results revealed that 2 mg l^{-1} (P3) was the best concentration of BAP to be added to the media to regenerate katak porang tubers of Porang Madiun 1 by in vitro.

1. Introduction

Porang (*Amorphophallus muelleri* Blume) or Iles-iles is one of bulbous plant from the *Araceaea* family which received a lot of attention and become famous among farmers recently. Porang is a food crop commodity that has high economic value. The high demand for porang increasing its exports to some countries such as Japan, China, Vietnam, Taiwan, Myanmar, and Thailand. According to [1] on July 2020, the shipments of porang commodities through agricultural quarantine increased to 160% from 2019. On July 2020 the shipments of porang reached Rp. 2.7 billion especially from Flores. Even the total value of porang exports from various regions in 2020 reached more than Rp. 1.3 trillion. The Minister of Agriculture enacting Porang as one of commodity that is included in the three-fold expo (Gratieks) movement because its export increasingly [2]. Porang is not only having high economic value, but also can be used as functional food ingredients because its contain of high fiber [3]. Porang tubers also contain glucomannan compounds [4] which according to [5] has benefit as cholesterol-lowering, anti-inflammatory [6], and anti-diabetic [7]. Porang tubers can be used as materials in food, cosmetic and pharmaceutical industries [2].

Porang plants grow well in tropics area, especially in Indonesia. This plant includes plants that are very easy to be cultivated and can grow well in the shade or direct sunlight. Porang planting can be done either using seeds or using stem tubers and katak/leaf bulbs [8]. However, porang tubers are mostly harvested before flowering. Harvesting after flowering can cause shrinking tubers and decrease its glucomannan

content. Thus seeds are not always available [9]. Planting with bulbs, bulbil, or leaf cuttings is constrained by dormancy problems so it takes long time. Another alternative is needed in porang propagation.

Micropropagation by tissue culture is an alternative method that can be used to reproduce porang plants. Micropropagation can produce plants faster, free season, in large quantities with relatively fast time. In addition, the seeds produced by tissue culture are better quality because they are uniform and free of disease [10]. One of the factors that mostly affect the success of propagation by tissue culture (in vitro) is the composition of the culture medium [11]. Growth regulators in the media can affect growth and morphogenesis in culture [12].

Growth regulators are one of the factors that determine the success of in vitro plant regeneration. Growth regulators that used to regenerate plants from the formation of shoots are cytokinins. Several types of cytokinins that can be used are Kinetin, Zeatin, 2l-P, Benzeladenine (BA), PBA and Thidiazuron (TDZ). According to [13], cytokinins added to culture media are generally to stimulate cell division, induce shoot formation and axillary shoot proliferation, but can inhibit root formation. The response of plant tissue/plant parts that were cultured was different to PGR auxins [14] and cytokinins [15], depends on the genotype (type and cultivar) of the plant [12]. Similarly, the response of plant cultivars to the concentration of growth regulators is determined by the genotype of the plant. The results of the research by [16] on 4 different tin cultivars (Poona Fig, Brown Turkey, Conadria and Deanna) and treated with different BAP concentrations (2.5 and 3.5 mg.l-1) showed the response of the four Tin cultivars depends on the genotype/cultivar of the plant. For this reason, this research is aim to observe the response of tuber explants to the application of several concentrations of BAP to regenerating Madiun 1 variety of porang from shoot formation/organogenesis. The research is aim to obtain the best concentration of BAP to regenerate Madiun 1 variety of porang from shoot formation (organogenesis).

2. Material and Methods

The research was carried out from June to October 2021, at Tissue Culture Laboratory of Politeknik Negeri Lampung. The materials used in this research were: Katak bulbs (bulbil) from Madiun 1 variety of Porang plant, chemicals (basic media Murashige and Skoog, 1962) supplemented with vitamins (thiamine-HCl, pyridoxine-HCl, nicotinic acid) and myo inositol, BAP growth regulators, as well as HCl and NaOH. Media-making materials include jelly, sugar, rubber, plastic, and aluminum foil. The tools used include autoclave, laminar air flow cabinet, hand sprayer, tweezers, scalpel, and petridish.

The experiment was carried out using complete randomized design with 7 treatment concentrations of BAP, that was : P1 (BAP 0 mg l^{-1}), P2 (BAP 1 mg l^{-1}), P3 (BAP 2 mg l^{-1}), P4 (BAP 3 mg l^{-1}), P5 (BAP 4 mg l^{-1}), P6 (BAP 5 mg l^{-1}), P7 (BAP 6 mg l^{-1}). Each treatment was consisted of 5 replications. The data were analyzed using Variety Analysis, and the differences between treatments were tested using the LSD test at the 5% level.

The research started with preparing media and explants. MS media was prepared by adding BAP with concentrations based on the treatments (0, 1, 2, 3, 4, 5, 6 mg.l-1)) ([17] on explants porang shoots (from seeds); [3] using explants iles-iles/porang leaf stalk; and [18] with tuber explants of Amorphophallus sp.). The pH of the medium was adjusted to 5.7 with the addition of HCl or NAOH. then the media was sterilized at 121 ◦C for 20 minutes. Sterile media are incubated for 3-5 days.

Porang tuber explants that have passed its dormancy period are grown in vitro. The tubers were washed and carefully soaped under tap water, then soaked in 2 g l^{-1} fungicide and bactericide each for 1 hour. Surfacing sterilization of explants was carried out by immersing the tubers in 50% bayclin solution for 30 minutes, and then the explants were removed and placed into the petridians. The tubers were peeled thinly and then soaked again in 10% bayclin for 10 minutes, and rinsed with sterile water 3 times. Furthermore,

the tubers were cut into quarters and planted in the treatment media 2 pieces per bottle. Subculture was carried out by removing tubers on 45 days after planting on the same treatment medium. Explants that have been planted and subcultured were maintained by placing culture bottles in ± 26 ◦C room temperature, with 16 hours of light and 8 hours of darkness. Observations for contamination were carried out every day. Contaminated culture bottles are immediately removed from the culture maintenance room.

Observation for budding was carried out by counting the number of spheres formed on explants with 2 mm of minimum diameter. The number of shoots was observed by counting all the shoots produced, with 5 mm of height. The shoot height (cm) was observed by measuring the shoots from the base to the tip of the highest shoot.

3. Results and Discussion

The initiation of culture was carried out using bulbs, in the form of katak bulbs or bulbils with 2.5-3 cm of diameter. The tubers were carefully washed under tap water and soaped, soaked with systemic fungicides and bactericides. Then sterilized in laminar.

Contamination is a major problem in growing porang tubers in vitro. To get sterile tubers, the tubers were sterilized using various methods of preparing tubers (peeled & unpeeled), concentration and duration of sterilization/soaking of tubers with sterilants. However, the results of sterilization obtained only 5-10% sterile tubers, while 90 to 95% contaminated. The main contaminants that cause tuber contamination are fungi (Figure 1), while bacterial contaminants reach 10-15% (Figure 2).

Figure 1. Contamination of tuber explants caused by fungus.

Figure 2. Contamination of tuber explants caused by bacteria.

Sterile bulbs began to respond on 15 days after planting by enlarging. Callus began to form on explants on 30 days after planting, from inside the cut tuber (Figure 3a). The texture of callus formed is little compact. Callus development can be seen on Figure 3.

In general, the addition of BAP to culture media can stimulate callus formation on explants of katak porang's tube pieces. The response of callus regeneration to be budding and shoot are different between benzyl amino purine concentrations treatments added to the media. The highest number of was obtained from explants cultured on media that was added 2 mg l^{-1} (P3) and 5 mg l^{-1} (P6) of BAP (Figure 4), followed by explants applied by 1/3/ 4/ 7 mg $l^{-1}$of BAP. The least number of shoots was produced on explants cultured on media without BAP (P1) (table 1). Explants cultured on media that were given 6 mg l^{-1} of BAP (P7) significantly produced better number of shoots than explants cultured on media without BAP or 1, 2, and 3 mg l^{-1} of BAP, shoots were produced on media treated with 4 mg l^{-1} (P5) and 5 mg l^{-1} (P6) of BAP did not significantly different. The highest growth of explants were cultured on media without the addition of BAP

(P1) (figure 5a). The addition 2 mg l^{-1} of BAP resulted the higher growth than the addition 3 mg l^{-1} of BAP. There was no differences in shoot height on explants cultured on media added 1, 4, 5, and 6 mg l^{-1} of BAP.

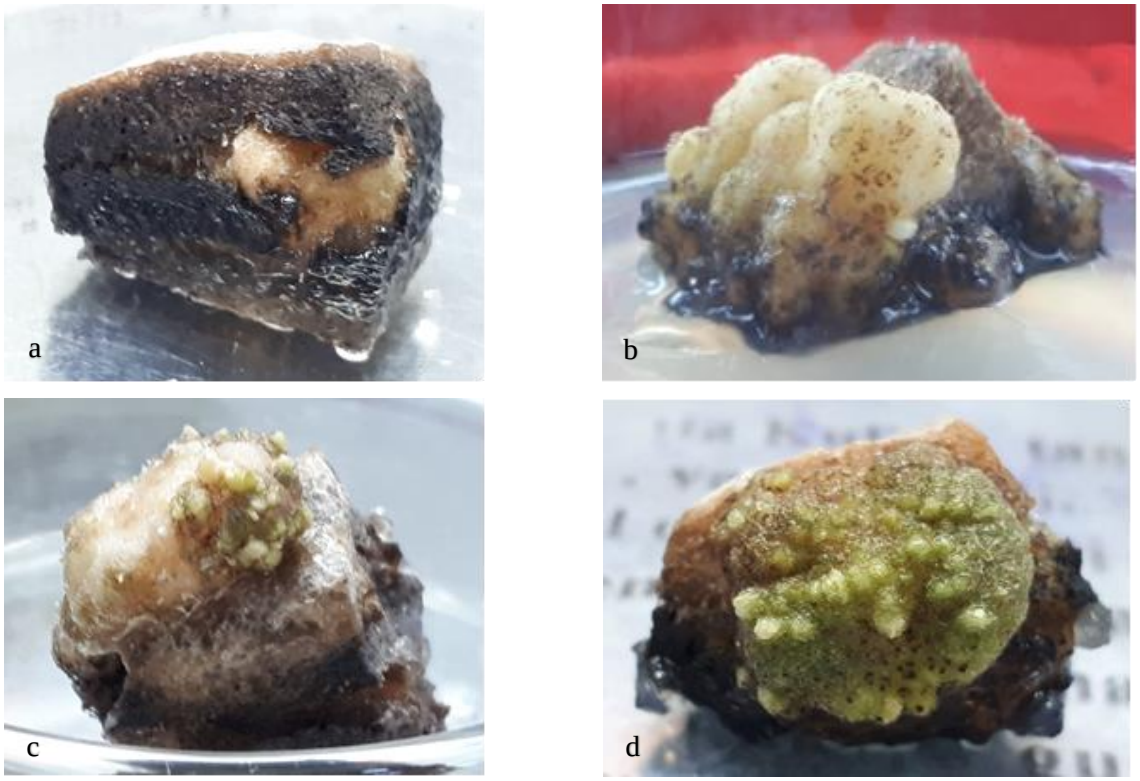

Figure 3. Callus development and growth of budding on explants of katak porang's tube pieces: initiation of callus on explant pieces on 30 days after planting (a), enlargement of callus groups out of tubers on 45 days after planting (b), initiation of budding on the callus surface on 60 days after planting (c), and the number and size of budding on the callus surface getting increase by the time (d).

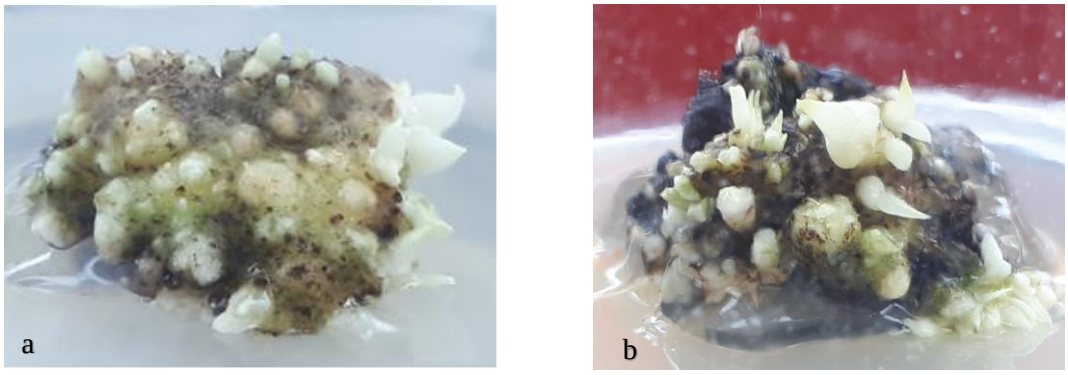

Figure 4. Growth of budding and shoots on explants treated with BAP: budding and shoots on explant with P3 media/BAP 2 mg.l^{-1} (a) and budding and shoots on explants with p6 media/BAP 5 mg.l^{-1} (b)

Table 1. Average number of shoots, shoot height, and number of porang buds in BAP concentration treatments.

Treatment	Average Number of Budding ± SD	Average Number of Shoots ± SD	Average Shoot Height (Cm) ± SD
P1 : (BAP 0 mg l^{-1})	3.4000 C	1.2000 D	1.0220 A
P2 : (BAP 1 mg l^{-1})	26.2000 B	2.6000 C	0.7380 B C
P3 : (BAP 2 mg l^{-1})	34.8000 A	4.0000 B	0.7537 B
P4 : (BAP 3 mg l^{-1})	26.0000 B	4.0000 B	0.6507 C
P5 : (BAP 4 mg l^{-1})	25.8000 B	4.8000 A B	0.7326 B C
P6 : (BAP 5 mg l^{-1})	32.2000 A	4.6000 A B	0.6963 B C
P7 : (BAP 6 mg l^{-1})	25.8000 B	5.8000 A	0.6826 B C

Note: The average number of budding, number of shoots, and shoot height followed by the letter the same results showed that it was not significantly different from the LSD test at the 5% level.

The fastest and highest shoot growth (Figure 5a) occurred in P1treatment (without the addition of BAP), although the number of budding and shoots produced were little. This is presumably that without giving BAP to the growth media, budding growth upwards (high growth), while with BAP the growth was to the side (multiplication of prospective shoots occurs in explants). This is in accordance in banana propagation, to duplicate the buds on banana propagules at the multiplication stage, the media was added by cytokinins (BAP), while for the banana shoot growth stage, the media was no longer added by BAP [10]. The growth of shoots (bud proliferation) and shoot height produced in explants without BAP or in explants that produced many buds on BAP-treated media (Figure 5).

From the results of the Tukey's HSD test on Table 1, can be seen that the addition of BAP to the media explant of porang katak tuber could induce callus formation on the explants of porang katak tuber. Application of BAP can also increase callus regeneration to form budding and increase the proliferation of budding into shoots. Benzyl Amino Purine is a cytokinin growth regulator which is widely used to stimulate shoot doubling. According to [19] cytokinins play a role in morphogenesis, such as inducing the formation of shoot organs. The strong activity of BAP [20] has led it to widely use to stimulate shoot multiplication in vitro. On the three variables observed (Table 1), the application 2 mg l^{-1} (P3) and 5 mg l^{-1} (P6) of BAP was the best concentration to regenerate katak tubers of porang by indirect organogenesis. This can be seen from the number of potential shoots produced on explants, although the number of shoots produced by the explants and the shoot height obtained the second best results. By subculture to the shoot elongation stage, it is possible to produce more shoots than other treatments. This needs to be studied more at a further stage, both proliferation and multiplication of these buds for propagation, because in research is not yet known the growth rate (number and height) of budding. Based on the results about the response of explants that applied by 2 mg l^{-1} (P3) and 5 mg l^{-1} (P6) of BAP at the same number of budding, number of shoots, and shoot height, it can be concluded that the best concentration for regenerating Porang Madiun 1 was the treatment with the addition 2 mg l^{-1} of BAP (P3).

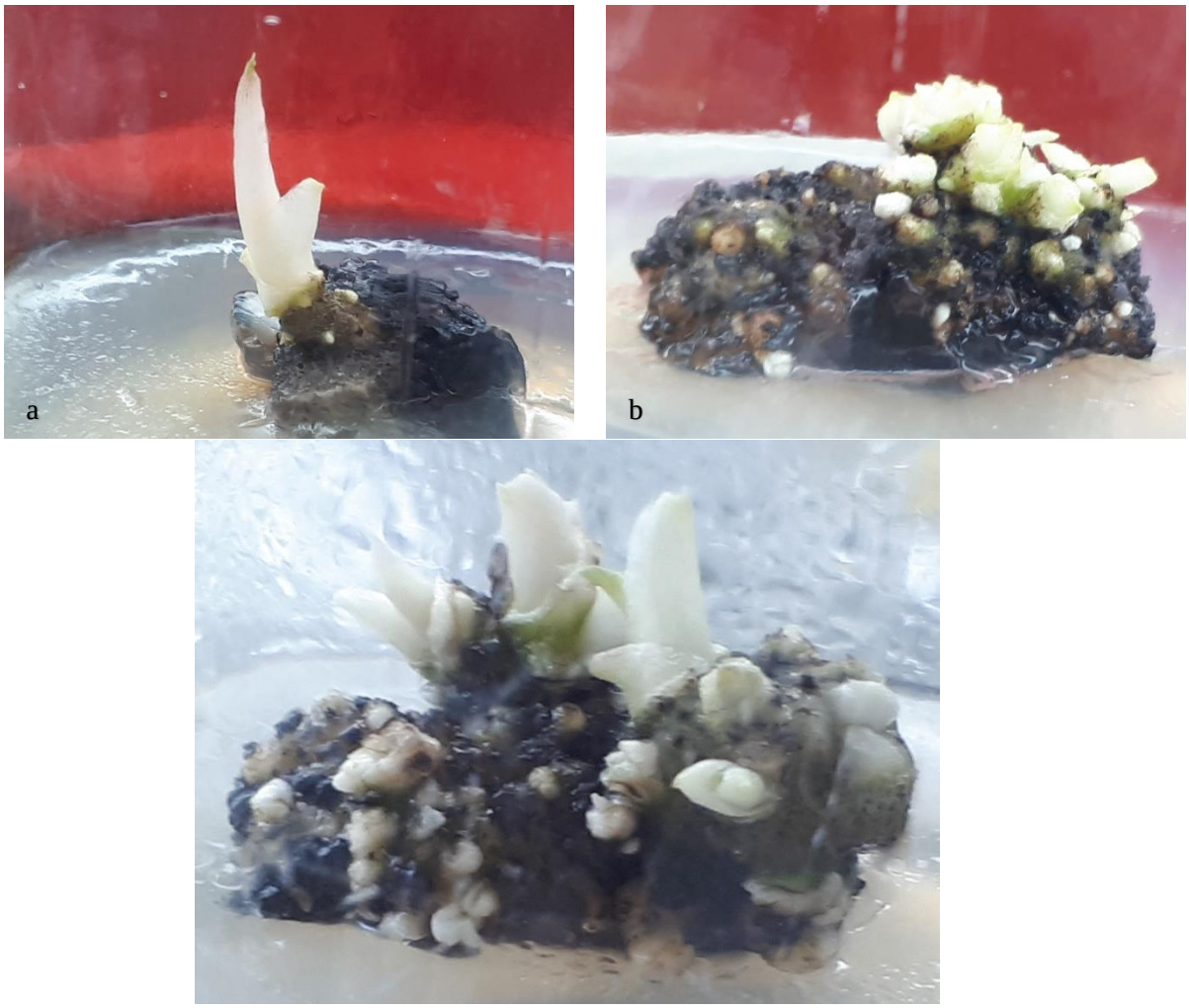

Figure 5. Growth of shoots on explants cultured on media without BAP and on media provided by BAP: growth of shoot height on explants cultured on P1 medium/0 mg l^{-1} (a), growth of shoot height on explants cultured on P7 media/BAP 6 mg l^{-1} (b), and growth of shoot height on explants cultured on P7 BAP 6 mg l^{-1} media (c).

4. Conclusion

The best concentration of BAP to be added to the media to regenerate the katak tubers of the Porang Madiun 1 was 2 mg l^{-1} (P3).

References

[1] Agrofarm 2020 Indonesia's Porang Exports Increase up to 160 Percent https://www.agrofarm.co.id/2020/08/26495/.

[2] Bandono S 2021 Ministry of Agriculture Prepares Porang Development Strategy as a 'Mahkota' Commodity http://technology-indonesia.com/pertanian-dan-pangan/inovasi-pertanian/kementan-siapkan-strategi-pengembangan-porang-sebagai-komoditas-mahkota/.

[3] Maria I, Wulansari A and Yuyu P S 2008 Regenerasi tunas dari kultur tangkai daun iles-iles (*Amorphophallus muelleri* Blume) *Biodiversitas* **9** 173–176 https://biodiversitas.mipa.uns.ac.id/D/D0903/D090304.pdf.

[4] Yohanes M, Tanto H and Novita D 2017 Pengaruh pemberian tepung porang (*Amorphophallus muelleri* Blume) terhadap kadar ureum pada tikus (*Rattus novergicus*) strain Wistar DM tipe 2 *J. Nurs. News*, **2** 665–667

[5] Zhang M, Sun L, Zhao W, Peng X, Liu F, Wang Y, ... and Zhou Y 2014 Cholesteryl-modification of a glucomannan from bletilla striata and its hydrogel properties *Molecules* **19** 9089–9100 doi: 10.3390/molecules19079089

[6] Zheng Q, Li W, Liang S, Zhang H, Yang H, Li M and Zhang Y 2019 Effects of ultrasonic treatment on the molecular weight and anti-inflammatory activity of oxidized konjac glucomannan *CYTA - J. Food* **17**, 1–10 doi: 10.1080/19476337.2018.1541195

[7] Kumar D S C P, Lokesh T, Gobinath M and Kumar B 2013 Anti-diabetic and anti-hyperlipidemic activities of glucomannan isolated from *Araucaria cunninghamii* seeds *J. Chem. Pharm. Sci*.**6**, 204–209

[8] Sumarwoto 2005 Iles-iles (*Amorphophallus muelleri* Blume); description and other characteristics *Biodiversitas J. Biol. Divers.* **6** 185–190 doi: 10.13057/biodiv/d060310

[9] Aziz M M, Ratnasari E and Rahayu, Y S 2014 Bulb callus induction of iles-iles (*Amorphophallus muelleri*) with combination concentration of 2,4-D and BAP by in vitro *LenteraBio*. **3** 109–114

[10] Sulistiani S A and Yani 2012 *Production of Plant Seeds Using Tissue Culture Techniques* Bogor: SEAMEO BIOTROP

[11] Widiyanto S 2011 *Micropropagation in Plant Science and Biotechnology.* Bandung: Scientific Speech Papers of Professors of the Bandung Institute of Technology

[12] Gunawan L W 1992 *Plant Tissue Culture Techniques. Ministry of Education and Culture*. Bogor: Directorate General of Higher Education, Inter-University Center for Biotechnology IPB

[13] Purnomo R, Santoso M and Heddy S 2013 Effect of various organic and inorganic fertilizers on growth and yield of cucumber (*Cucumis sativus* L.) *J. Produksi Tanam.* **1**, 93–100

[14] Firoozabady Y, Moy E 2004 Regeneration of pineapple plants via somatic embryogenesis and organogenesis *Vitr. Cell Dev. Biol. Plant* **40** 67 – 74

[15] Sripaoraya M R, Marchant S, Power R and Davey J D 2003 Plant regeneration via somatic embryogenesis and organogenesis of commercial pineapple (*Ananas comosus* L.) *Vitr. CellDev. Biol. Plant* **39** 450–454.

[16] Dhage S S, Chimote V P, Pawar B D, Kale A A, Pawar S V and Jadhav A S 2015 Development of an efficient in vitro regeneration protocol for fig (*Ficus carica* L.) *Journal of Applied Horticulture* **17** 160–164

[17] Suheriyanto D, Romaidi and Resmisari R S 2012 Pengembangan bibit unggul porang (*Amarphopallus oncophilus) melalui teknik kultur in vitro untuk mendukung ketahanan pangan* nasional *El-Hayah* 3, 16–2

[18] Thach B D, Linh N K, Duy T T B, Giang T T L, Uyen N P A, Suong N K and Van Du N 2016 Preliminary selection and in vitro propagation of glucomannan distributed in Vietnam *Eur. J. Adv. Res. Biol. Life Sci*.**4** 1–7

[19] Wiraatmaja I W 2017 *Gibberellins and Cytokinins Growth Regulators* Bali

[20] Zaer and Mapes 1982 *Action of growth regeneration In. Bonga and Durzan (eds.)* London