MICROPROPAGATION OF *Amorphophallus titanum* Becc. (ARACEAE)

Perbanyakan Mikro Amorphophallus titanum Becc. (Araceae)

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Abstrak

Amorphophallus titanium Becc. atau bunga bangkai memperbanyak diri secara alami melalui bijinya, tetapi karena pemasakan bunga betina dan jantan tidak bersamaan waktunya, maka jarang dijumpai buah/bijinya. Perbanyakan secara vegetatif diharapkan dapat dimanfaatkan untuk mempertahankan keragaman genetiknya. Eksplan dari tunas samping umbi *Amorphophallus titanum* yang berasal dari Lembah Anai, Sumatera Barat ditumbuhkan pada media Murashige-Skoog (MS) and Gamborg B5 dengan penambahan 0,1 mg/l NAA and 1 mg /lBA, dengan dan tanpa 1 mg/l 2,4-D atau 0,1 mg/l NAA dan 0,01 mg/l Kinetin. Penambahan arang aktif tidak mempengaruhi pertumbuhan tunas dan daun. Selanjutnya kultur yang ditumbuhkan pada media MS dengan penambahan berbagai kadar *trans*-Cinnamic Acid (t-CA) dan Kinetin dapat menumbuhkan tunas tunggal ataupun ganda dari pucuknya. Kultur yang berasal dari kalus, akar atau potongan pelepah daun pertumbuhan tunasnya terbatas. Media terbaik untuk pertumbuhan tunas akar dan umbi pada media mengandung 0,3 mg/l NAA dan 0,03 mg/l Zeatin.

Kata kunci: *Amorphophallus titanum*, tunas aksial, perbanyakan mikro.

INTRODUCTION

Amorphophallus titanum Becc. produces the most ashtonishing inflorescence and leaves in the plant kingdom. The plants produce flower only on reaching maturity. Seeds are produced rarely under cultivation without artificial pollination. From several records, Odoardo Beccari who discovered in Sumatra in 1878 and he sent several tubers and some seeds to M. Salviati in Italy but the tuber rotted and one or some seedlings were sent to Kew. After 11 years of cultivation, one plant flowered, but this plant died 2 years after flowered (Gandawidjaja *et al.*, 1983; Lobin *et al.*, 2009). Prana (2000) germinated *A. titanium* seeds and found that embryoless seed portion able to produce roots and shoots. Further trials, 6-benzyl amino purine (BAP) was added to the germinating medium, but there was no different effect on their germination rate (Prana, 2001).

Leaf cuttings is another propagation mehod as described by Upton in Lobin *et al.*, 2009). Propagation of *A. titanium* to reduce pressure on natural population was initated in Bogor Botanic Garden and Royal Botanic Gardens Sydney. The propagation material from Cibodas Botanic Garden. Unfortunately this collaboration was discontinued (Perrins, 2002).

Efforts to propagate *A. titanum* were initiated using a method used for *A. paeoniifolius* (*A. campanulatus* var. *hortensis*) since this method had been proved had a good result (Irawati *et al.*, 1986).

Other species in the genus as example *Amorphophallus rivieri* and *A. konjac,* have been propagated successfully through tissue culture (Asokan *et al.*, 1984; Shimoyama, 1986). Kohlenbach and Becht (1988) succesfully regenerated plants from midveins of *A. titanum* and *A. rivieri.* They produced plantlets which formed tubers on solid medium. Their result showed that one of the main constrains in tissue culture of *A. titanum* was obtaining sterile explants. *In vitro* propagation on *Amorphophallus albus* by Hu and Li (2008) showed that two morphogenetic pathways, namely adventitious bud and corm-like structure were observed on organogenic of the callus. NAA and BA added to MS medium produced adventitious buds as well as corm-like structure and these morphogenetic events originated from the meristematic cells located in superficial layers of the callus tissue. To increase corm formation for the same species, Hu *et al.* (2006) added sucrose from 2% to 6% and the result significantly increased the corm formation rate and favoured corm growth but gave negative effects at higher sucrose concentration.

This study was undertaken for the purpose formulating the optimal medium for micropopagation of *A. titanum*. Such a method would be of considerate importance in the conservation of this species.

MATERIAL AND METHODS

Corms were collected from West Sumatra. The corm was first washed with a brush and running water to remove soil and debris. Proper washing before sterilization is critical for contamination prevention. Sections of corm (1 cm^3) containing lateral buds were surface sterilized in 10% Clorox for 20 minutes, in 5% Clorox for 10 minutes, both sterilants contained a few drops of Tween 80 and 2.5 mg/l Ascorbic acid. After that the corm were rinsed in sterile Ascorbic acid solution three times. The bracts which cover the buds were removed after this and the lateral buds were incised in cubes of 0.5X0.5X0.5 mm under a binocular microscope. These cubes were placed on the medium with additional auxin and cytokinin had been used for other aroids *(Amorphophallus paeoniifolius, A. rivieri, Colocasia esculenta*) before (Table 1).

Table 1. Composition of media for *Amorphophallus titanum* initiation.

Basal medium	Sucrose g/l	Activated charcoal g/l	Agar g/l	NAA mg/l	BA mg/l	Kinetin mg/l	2,4 - D mg/l
MS	30	2	10	-	-	-	-
MS	30	2	10	0.1	1	-	-
MS	30	2	10	0.1	1	-	1
MS	30	2	8	0.1	-	0.01	-
Gamborg B5	30	2	8	0.1	-	0.01	-

Note: MS = Murashige & Skoog medium pH was adjusted to 5.3-5.9.

The cultures were transplanted every 6 weeks to the same medium and used for further studies. Shoot tips, young petioles, midrib, leaf cut and callus from corm were subsequently cultured on the multiplication medium. Six different multiplication media (Table 2) were tested for suitability to *Amorphophallus titanum* explants. All cultures producing shoot/s were transplanted in MS medium with vitamins and 10 mg/l 2-iP.

Table 2. Multiplication media for *Amorphophallus titanum* tissue culture.

Basal medium	Sucrose g/l	Activated charcoal g/l	NaH2PO4 mg/l	Adenine Sulphate mg/l	IAA mg/l	Kinetin mg/l	BA mg/l	Thiamin e mg/l
MS	30	-	170	80	2	2	-	0.4
MS	30	2	170	80	2	2	-	0.4
MS - v	45	-	85	80	0.5	10	-	-
MS - v	45	2	85	80	0.5	10	-	-
MS - v	30	-	-	-	-	-	1	-
MS - v	30	2	-	-	-	-	1	-

Note: MS = Murashige & Skoog medium with vitamins (10 mg/l Nicotinic acid + 30 mg/l Thiamine HCl. + 10 mg/l Pyridoxin HCl. + 100 mg /l L-tyrosine)
MS – v = Murashige and Skoog without vitamin All at pH 5.5

Sixteen different media containing Kinetin and *trans*-Cinnamic Acid were tested for their ability to induce shoots (Table 3). Different explants (white and green) callus, callus with produced shoots with thick meristemoids bracts, slices of large roots and petioles were used to initiate the culture. Each treatment was replicate 10 times.

Table 3. Composition of media for *Amorphophallus titanum* shoot induction.

Basal medium	Sucrose g/l	Agar g/l	Kinetin mg/l	*trans*-Cinnamic Acid mg/l	pH
MS	30	8	0	0	5.5
MS	30	8	0	0.1	5.5
MS	30	8	0	0.5	5.5
MS	30	8	0	1.0	5.5
MS	30	8	0.1	0	5.5
MS	30	8	0.1	0.1	5.5
MS	30	8	0.1	0.5	5.5
MS	30	8	0.1	1.0	5.5
MS	30	8	1.0	0	5.5
MS	30	8	1.0	0.1	5.5
MS	30	8	1.0	0.5	5.5
MS	30	8	1.0	1.0	5.5
MS	30	8	5.0	0	5.5
MS	30	8	5.0	0.1	5.5
MS	30	8	5.0	0.5	5.5
MS	30	8	5.0	1.0	5.5

Several cytokinins were also tested for their ability to induce shoots from callus (Table 4). Higher concentration of hormones in shoot induction media without activated charcoal was also tested (Table 5).

Table 4. Shoot induction media.

Basal medium	Sucrose g/l	Activated charcoal g/l	Myo-Inositol mg/l	Agar g/l	NAA mg/l	Kinetin mg/l	BA mg/l	2-iP mg/l	Zeatin mg/l	pH
MS	30	2	100	8	0.1	0.1	-	-	-	5.35
MS	30	2	100	8	0.1	-	0.1	-	-	5.57
MS	30	2	100	8	0.1	-	-	0.1	-	5.40
MS	30	2	100	8	0.1	-	-	-	0.1	5.35

Table 5. Shoot induction media.

Basal medium	Sucrose g/l	NaH2PO4. 2H2O mg/l	Thiamine HCl mg/l	Adenin Sulphate mg/l	NAA mg/l	2-iP mg/l	Kinetin mg/l	Zeatin mg/l	pH
MS	30	192	0.4	71.59	0.3	30	-	-	5.7 2
MS	30	192	0.4	71.59	2.0	-	2	-	5.7 2
MS	30	192	0.4	71.59	1.0	-	-	0.02	5.7 8

A last series of media was used to compare the effect of coconut water, different cytokinins in combinations with a standard concentration of NAA and anti-auxin (Table 6). Explants were taken from corms, callus, shoot tips and petioles.

Table 6. Shoot induction media.

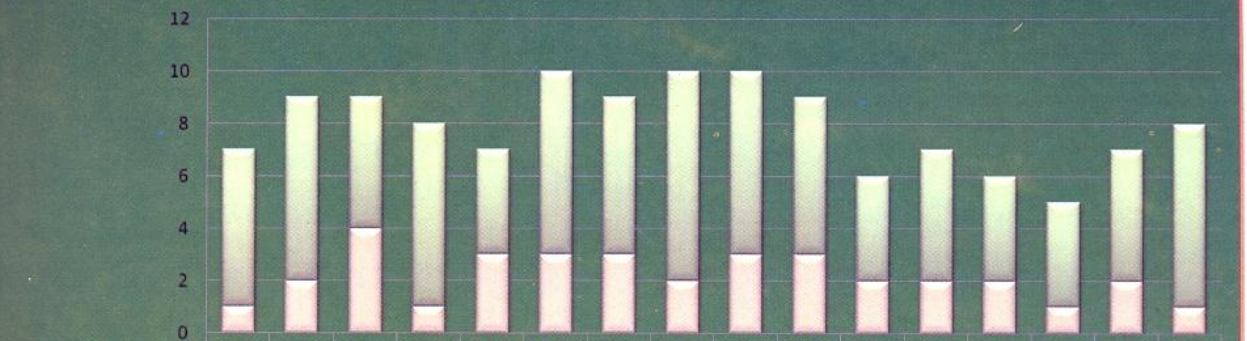
Basal medium	Sucrose g/l	Gelrite g/l	CW ml/l	NAA mg/l	2-iP mg/l	Kinetin mg/l	Zeatin mg/l	BA mg/l	2,4-D mg/l	t-CA mg/l
MS	30	2	-	-	-	-	-	-	-	-
MS	30	2	150	-	-	-	-	-	-	-
MS	30	2	-	0.3	3	-	-	-	-	-
MS	30	2	-	0.3	-	1	-	-	-	-
MS	30	2	-	0.3	-	-	0.03	-	-	-
MS	30	2	-	0.3	-	-	-	3	-	-
MS	30	2	-	-	-	-	-	-	0.03	-
MS	30	2	-	-	-	-	-	-	-	3

RESULTS AND DISCUSSION

The explant tend to turn brown due to production of phenolic compounds, treatment with the Ascorbic Acid solution reduce browning. Original explants in five different inoculating media (Table 1) became larger. Some of them (<10%) produced shoots with or without root, others became swollen and formed compact calli (> 90%). There was no significant differences development of the culture on the media tested.

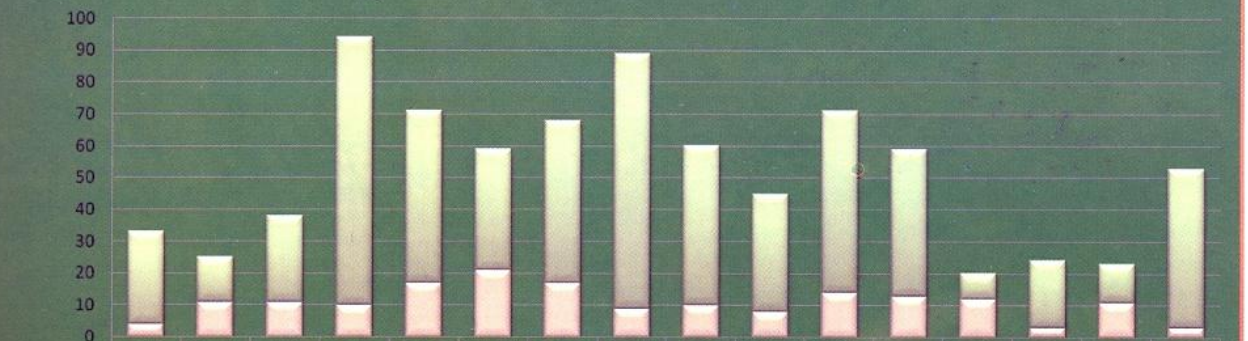
Multiplication media (Table 2) showed that the addition of activate charcoal to different media also did not improve the multiplication of the cultures. Not all culture producing shoot and root, some cultures only enlarged others producing single or multiple shoot/s with or without root. The number of culture producing shoot/s and root/s in each treatments and the total number of shoot/s and root/s is shown in Figure 1 and 2.

Figure 1 : Number of Culture with Shoot and Root



t-CA - Kinetin (mg/l)	0-0	0-0.1	0-1	0-5	0.1-0	0.1-0.1	0.1-1	0.1-5	0.5-0	0.5-0.1	0.5-1	0.5-5	1-0	1-0.1	1-1	1-5
no. culture with shoot	6	7	5	7	4	7	6	8	7	6	4	5	4	4	5	7
no culture with root	1	2	4	1	3	3	3	2	3	3	2	2	2	1	2	1

Figure 2 : Total Number of Shoot and Root



t-CA - Kinetin (mg/l)	0-0	0-0.1	0-1	0-5	0.1-0	0.1-0.1	0.1-1	0.1-5	0.5-0	0.5-0.1	0.5-1	0.5-5	1-0	1-0.1	1-1	1-5
total shoot	29	14	27	84	54	38	51	80	50	37	57	46	8	21	12	50
total root	4	11	11	10	17	21	17	9	10	8	14	13	12	3	11	3

Figure 1 shows that effect of Kinetin and t-CA on the number of culture producing shoot and root. The result showed that the number of culture producing shoot and root did not significantly different for the treatments tested, but the number of culture producing shoot/s higher in medium containing high concentration of Kinetin. The calli grew into green and white compact callus masses, some (approximately 10%) became vitreous and did not grow further (Figure 3). Shoot and roots developed on approximately 30% of the cultures. In most cases shoot development was followed by roots i.e. in 35% of the cultures, and later was followed by rapid leaf growth.

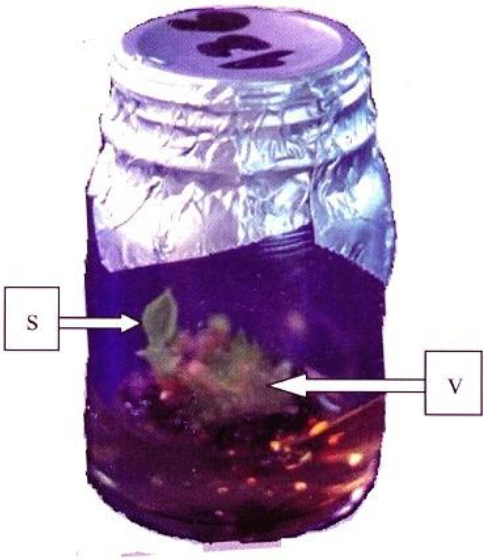


Fig. 3. *Amorphophallus titanum* culture with a normal shoot (s) and vitrous abnormal shoots (v)

Figure 2 shows that the total number of shoot high in low concentration of anti auxin: *trans*-Cinnamic Acid, but most of the shoot are stunted.

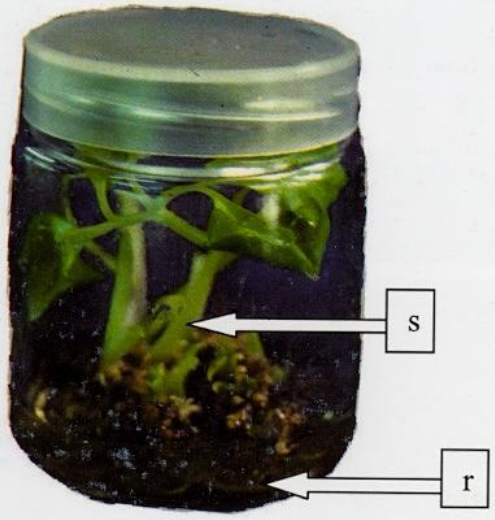


Fig. 4. *Amorphophallus titanium* culture with shoots (s) and roots (r).

Root development did not show differences between treatments and relatively limited, but the roots in most cultures are normal roots (Figure 4).

Different types of explants have different growing ability in eight shoot inducing media tested. Between 6-15% of a total 90 shoot tip explants developed into plantlets with the exception of media with Kinetin and BA. Only 0.5-6.0% of explants from corm producing plantlets, 0.6-3.8 % producing roots and 38-60% of the cultures only enlarged.

The best medium for *Amorphophallus* explants is MS + 0.3 mg/l NAA + 0.03 mg/l Zeatin, followed by MS + 0.3 mg/l NAA + 3 mg/l 2-iP and MS + 150 ml/l coconut water. Plantlet developed from different types of explants producing one or more leaves. The plantlet has normal roots but none of them have normal corms (Figure 5), all corms have irregular shapes. Later the plantlets were established well at the nursery, some of them with more than one leaf (Figure 6).

Fig. 5. Seedlings with corm, roots and a shoot.

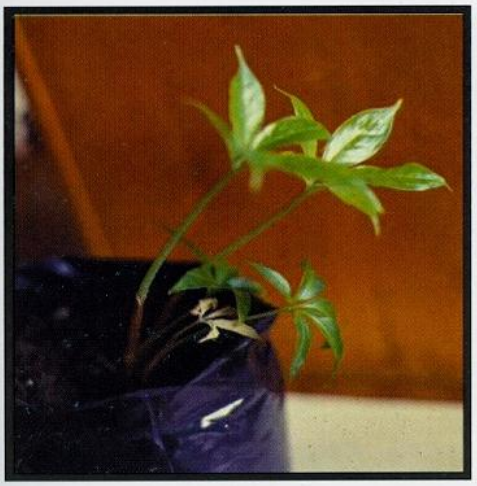


Fig. 6. Multiple shoots seedling in the nursery.

Naphthalene Acetic Acid (0.3 mg/l) and Zeatin (0.03 mg/l) are suitable for developing *Amorphophallus titanum* plantlet as well as for *A. rivieri* (Asokan *et al.,* 1984), but different from *Amorphophallus paeonifolius* (*A. campanulatus* var. *hortensis*) which showed the best result for plantlet development with additional 0.1 mg/l NAA and 0.01 mg/l Kinetin to the medium. Similar result for *Amorphophallus titanum* and *A. paeoniifolius* were found for additional of coconut water to the medium (Irawati *et al.*, 1986). In most cultures, meristemoid shoots were also found, as characteristic form of cultures shown by *A. rivieri* (Asokan *et al.,* 1984).

Percentage of plantlet development is relatively low, however plantlet could be induced from the callus. Previous works by Kohlenbach and Becht (1988) on mid-vein explants of *A. titanium* and *A. rivieri* grown on MS medium + 2,4-D + Kinetin and from this study proved that undetected endogenous plant hormone/s play an important role in the development of cultures. Most seedlings have irregular shaped of corm, therefore cell cultures is suggested for further study.

CONCLUSION

A method to propagate *Amorphophallus titanum* had been established. Obtaining sterile explants from tuber is the key of success during inoculation. Ascorbic acid was proved as an effective substance to reduce browning during inoculation. Further development of the cultures had not been showed a significant differences with the additional of auxins, cytokionins as well as anti auxin tested. Further study using different auxins, cytokinins, antiauxins, organic compounds at different concentrations as well as initiating culture from other part of the plant such as leaf vein, lamina or petiole is recommended.

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