The effects of benzil amino purin (BAP) and gibberellin with in vitro seedling growth of pulesari (*Alyxia reinwardtii* Bl)

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Abstrak

Latar belakang: *Status kelangkaan pulesari (Alyxia reinwardtii Bl.) termasuk rawan (vulnerable) karena tingginya permintaan dan mahalnya harga menyebabkan eksploitasi pulesari di hutan meningkat sedangkan upaya konservasi dan budidayanya belum ada. Selama ini perbanyakan pulesari hanya mengandalkan biji di alam. Benih pulesari persentase perkecambahannya tergolong sangat rendah, waktu lama dan sulit diperbanyak secara vegetatif. Memperhatikan sulitnya mendapatkan bibit pulesari secara konvensional maka perlu dilakukan dengan cara kultur jaringan.*

Metode: *Penelitian dilakukan di laboratorium kultur jaringan Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional Tawangmangu selama tiga bulan. Bibit A. reinwardtii Bl. diperoleh dari Pringgondani Forest, Tawangmangu. Biji kupas dan dikeringkan selama lima hari, dicuci menggunakan aquadest steril, direndam pada 0,5% Agrept 5 menit kemudian pada 2,125% natrium hipochorida 5 menit dan dibilas menggunakan aquades steril. Media Murashige dan Skoog (MS) disiapkan sesuai komposisi. Penelitian ini rancangan acak lengkap (RAL) pola faktorial. Faktor pertama konsentrasi zat pengatur tumbuh BAP pada konsentrasi 3, 4 dan 5 mg/l dan faktor kedua konsentrasi giberelin dari 1, 2 dan 3 mg/l. Media MS yang dimodifikasi dengan penambahan BAP konsentrasi 3, 4, dan 5 mg/l dan giberelin konsentrasi 1, 2, dan 3 mg/l sehingga diperoleh 9 kombinasi perlakuan.*

Hasil: *Hasil penelitian menunjukkan bahwa kombinasi perlakuan zat pengatur tumbuh BAP mg/l dan giberelin 2 mg/l pada media MS terbentuk tunas dan akar. Tunas terbentuk pada 30 hari setelah tanam dengan tinggi 2 cm dan akar muncul pada 45 hari setelah tanam dalam satu kali percobaan.*

Kesimpulan: *Pulesari (Alyxia reinwardtii Bl.) dapat diperbanyak melalui kultur jaringan menggunakan biji sebagai eksplan.* ***(Health Science Journal of Indonesia 2016;7(2):93-6)***

Kaca kunci: *pulesari, Alyxia reinwardtii Bl, BAP dan giberelin*

Abstract

Background: *Alyxia reinwardtii* Bl. in natural habitat becomes rare and reaches vulnerable status. The high price and high market demand made the over harvested beyond regeneration capacity of the plant while the conservation efforts are terribly limited. Presentage of seed germination is not only very low but also time consuming, therefore we conducting preliminary study on in vitro propagation using seed *Alyxia reinwardtii* Bl.

Methods: The research was conducted in laboratory tissue culture of Medicinal Plant and Traditional Medicine Research and Development Center Tawangmangu for three months. Seeds of *A. reinwardtii* Bl. obtained from Pringgondani Forest, Tawangmangu Central Java. Seeds were peeled and air dryed for five days, washed using steril aquadest, soaked on 0.5% Agrept (5 minutes) then on 2.125% sodium hypochloride (5 minutes) and rinsed using sterile distilled water. This study uses a Murashige and Skoog (MS) with a completely randomized design (CRD) factorial design.. The first factor is BAP at a concentration of 3, 4, 5 mg.L-1 and the second factor is giberelin concentration of 1, 2, 3 mg.L-1. MS media with addition of BAP the concentration of 3, 4, 5 mg.L-1 and gibberellin the concentration of 1, 2, 3 mg.L-1 obtained 9 treatment combination.

Results: The results showed the combination treatment of growth regulators BAP (4 $mg.L^{-1}$) and giberelin (2 $mg.L^{-1}$) is formed shoots and roots. The shoots were formed 30 days after planting with a height of 2 cm and the roots appear at 45 days after planting.

Conclusion: Pulasari (*Alyxia reinwardtii* Bl.) can be propagated through tissue culture using seeds as explants. ***(Health Science Journal of Indonesia 2016;7(2):93-6)***

Keywords: pulesari, *Alyxia reinwardtii* Bl, BAP and giberelin

Pulesari (*Alyxia reinwardtii* Bl.) is one of medicinal plants which often used as main ingredient of Jamu, an Indonesia traditional medicine. The utilization of *A. reinwardtii* Bl. is usually accompanied by 'adas' (fennel, *Foeniculum vulgare* Miller), hence the name *'adas-pulasari'*. At least about 18 sorts of manufactured 'jamu' from Central Java - Indonesia contain 'adas-pulasari' as a principle ingredient. These are used as an antispasmodic and as treating of stomach-ache, flatulence, colic, fever, dysentery, as a carminative and sprue.[1]

The scarce status of *A. rewardtii* Bl. is because of its high market demand followed by its high price of the simplicia. These situations lead to the increasing of plant exploitation from the natural habitat, whereas neither any efforts on conservation nor cultivation.[2]

Up to present time, propagation of *A. rewardtii* Bl. relies on the seeds obtained from the wild. Unfortunately, pulesari seeds germination are very low and time consuming to yield mass seedling production. Besides having uncertain flowering behavior, its low growing rate play a significant role on decreasing of the existance of the plant.[3]

Plant tissue culture technology is a mainstay technology in the biodiversity utilization in Indonesia. The technique generates high multiplication and provides rapid production of many genetically identical plants with relatively small amounts of space, supplies and time. These methods eliminate genetic retardation due to unintentional mistakes during conventional seeding production.[4,5]

The success of tissue culture of plant propagation is influenced by planlet and culture medium. It also depends on the addition of plant hormones and growth regulators into the medium. Plant hormones are group of unrelated chemical substances that affect plant morphogenesis. Five major plant hormones are traditionally described: auxins, cytokinins, gibberellins, ethylene, and abscisic acid.[6,7]

Auxin and cytokinin play fairly important roles in many aspects of plant growth and development. The interaction between auxin and cytokinin is particularly important to control a few development processes, such as the formation and the maintenance of meristems that are essential to establish the whole plant body.[8] Cytokinin serves to stimulate cell division and proliferation of shoots. Cytokinin effects also include on breaking of dormancy, promotion of seed germination, stimulation and nutrient mobilization, enhancing anthocyanin and flavanoid synthesis, increasing resistance to disease, and stimulation of the opening of stomata.[9] Cytokinin influence on shoot initiation. Cytokinins influence the initiation of long shoots and buds. Cytokinins can also trigger the formation of side shoots, leaves widening and stimulate the formation of shoots.[10]

Giberelins in plant play an important role on stimulating the formation of flowers, pollens and seeds, enlarging the size of fruits, and terminating seed dormation. At low concentration gibberellin will not stimulate the root formation, otherwise at high concentration it will do. Gibberellin added for differentiation or multiplication cell function, especially the formation of callus. The use of gibberellin in the plant will affect plant height, root length size and leaf area. The most obvious effect of gibberellin is modifying plant growth, namely cell division that led to the increase in the number of cells and cell enlargement, so that root growth is faster and longer many.[11,12] Research concerning propagation and cultivation of *A. reinwardtii* Bl. in Indonesia still few, therefore *in vitro* propagation study of *A. reinwardtii* Bl. is needed. The research aimed to obtain breeding methods in tissue culture pulesari (*Alyxia reinwardtii* Bl.).

METHODS

The research was conducted in laboratory tissue culture of Medicinal Plant and Traditional Medicine Research and Development Center Tawangmangu for three months. Selected ripe seeds of *A. reinwardtii* Bl. which had dark brown husk were collected from Pringgondani Forest, Tawangmangu-Central Java. Seeds were peeled and air-dryed for five days, washed using steril aquadest (s.a), soaked on 0.5% Agrept (w/v) for 5 minutes then on 2.125% sodium hypochloride (v/v) for 5 minutes and rinsed three times using s.a. and placed on Laminar Air Flow (LAF) prior to planting. Murashige and Skoog (MS) media were prepared according to directed composition.[15] The research used a completely randomized design (CRD) factorial with two factors with 3 repetitions. The first factor was the concentration of growth regulators BAP at a concentration of 3, 4 and 5 mg.L-1 and the second factor was giberelin concentration

of 1, 2 and 3 mg.L-1. MS media were modified with addition of BAP with the concentration of 3, 4, and 5 mg.L^{-1} and gibberellin with the concentration of 1, 2, and 3 mg.L^{-1} with 3 repetitions.

All steril ambiance were done on LAF, media and utensils were sterilized using autoclave The eksplants were incubatated for 75 days, whereas the growth of the explants were examined regularly during incubation periode.

RESULTS

From the observation and measurement of *in vitro* seeding of *A. reinwardtii* Bl. showed that not all the treatment could give good results (table 1.)

From the observation and measurement of *in vitro* seeding of *A. reinwardtii* Bl. showed that not all the treatment could give good results (table 2.)

DISCUSSION

MS media enriched the combination of BAP 3 mg.L^{-1} with gebberellin 1, 2 and 3 mg.L^{-1}, combination of BAP 4 mg.L^{-1} with gebberellin 1and 3 mg.L^{-1} also combination of BAP 5 mg.L^{-1} with gebberellin 1 and 2 mg.L^{-1} had no significant effect yet on the growth of seed eksplant of *A. reinwardtii* Bl., it indicated that the concentrations were still under optimum conditions. While the addition of BAP 5 mg.L^{-1} and gibberellin 3 mg.L^{-1} only initiated root formation, it might the the concentration of BAP and gibberellin were too high. The presence of growth regulator will increase the growth and development of the plant if only it meet plant necessity, too high or too low doze will not give a positive effect. According to this examination, gibberellin at low concentration (<2 mg.L^{-1}) would not stimulate root formation.[14]

Table 1. Influence of Benzil Amino Purin (BAP) and Gibberellin to in vitro seeding of *Alyxia reinwardtii* Bl. at 75 days

Treatment	Shoot Initiation Time (day)	Shoot Length (cm)	Amount of Leaf	Root Initiation Time (day)	Amount of root
MS + BAP 3 mg.L^{-1} + Giberelin 1 mg.L^{-1}	n.a	-	-	n.a	-
MS + BAP 3 mg.L^{-1} + Giberelin 2 mg.L^{-1}	n.a	-	-	n.a	-
MS + BAP 3 mg.L^{-1} + Giberelin 3 mg.L^{-1}	n.a	-	-	n.a	-
MS + BAP 4 mg.L^{-1} + Giberelin 1 mg.L^{-1}	n.a	-	-	n.a	-
MS + BAP 4 mg.L^{-1} + Giberelin 2 mg.L^{-1}	45	4	2	30	1
MS + BAP 4 mg.L^{-1} + Giberelin 3 mg.L^{-1}	n.a	-	-	n.a	-
MS + BAP 5 mg.L^{-1} + Giberelin 1 mg.L^{-1}	n.a	-	-	n.a	-
MS + BAP 5 mg.L^{-1} + Giberelin 2 mg.L^{-1}	n.a	-	-	n.a	-
MS + BAP 5 mg.L^{-1} + Giberelin 3 mg.L^{-1}	n.a	-	-	45	1

Table 2. Influence of Benzyl Amino Purine (BAP) and Gibberellins on the growth of shoots and roots of *Alyxia reinwardtii* Bl at 75 days.

Treatment	Shoot Initiation	Root Initiation
MS + BAP 3 mg.L^{-1} + Giberelin 1 mg.L^{-1}	-	-
MS + BAP 3 mg.L^{-1} + Giberelin 2 mg.L^{-1}	-	-
MS + BAP 3 mg.L^{-1} + Giberelin 3 mg.L^{-1}	-	-
MS + BAP 4 mg.L^{-1} + Giberelin 1 mg.L^{-1}	-	-
MS + BAP 4 mg.L^{-1} + Giberelin 2 mg.L^{-1}	3	3
MS + BAP 4 mg.L^{-1} + Giberelin 3 mg.L^{-1}	-	-
MS + BAP 5 mg.L^{-1} + Giberelin 1 mg.L^{-1}	-	-
MS + BAP 5 mg.L^{-1} + Giberelin 2 mg.L^{-1}	-	-
MS + BAP 5 mg.L^{-1} + Giberelin 3 mg.L^{-1}	-	3

In this study, MS media enriched with the combination of BAP 4 mg.L^{-1} and gibberellin 2 mg.L^{-1} gainned the best result. Shoot and root initiation occurred at 30 and 45 days after planting respectively. The growth of shoot was 4 cm in length after 75 days of incubation. In this case the concentration of these growth hormones met with the need of *A. reinwardtii* Bl. seed in forming shoot and root. At appropriate concentration, cytokinin (BAP) plays as growth regulator which stimulates shoot formation. BAP stimulate plant cells to devide and differenciate in order to form organ. Cytokinin has an effect as shoot initiation. BAP (cytokinin) is able to enhance morphogenesis which increasing ell division, shoot formation and enhance meristematic cell to proliferate.[11] Media browning were seen around the seed of *A. rewardtii* Bl., it may happened because of the high content of essential oil of the seeds.

Gibberellin has an effect on growth and germination of the embryo. Gibberellins will stimulate amylase synthesis, this enzym will catalize amylum found in endosperm into glucose as source of energy for growing. A stanted plant will grow normally by adding of gibberellins. Gibberellins also terminate a period of seed dormantion. From the above result if can be concluded that shoot and root initiation time of seed of *Alyxia reinwardtii* Bl. on MS media enriched with BAP 4 mg.L^{-1} and gibberellin 2 mg.L^{-1} were 45 and 30 days after planting respectively. The best growth of shoot and root of *A. reinwardtii* Bl. seed could be obtained from MS media enriched with BAP 4 mg.L^{-1} and gibberellin 2 mg.L^{-1}.

In conclusion, pulasari (*Alyxia reinwardtii* Bl.) can be propagated through tissue culture by using seeds as explant.

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