

Effects of colchicine solution and LEDs treatment on survival rate and morphological characters of *Bulbophyllum auricomum* Lindl. *in vitro*

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Abstract

The purpose of this research was to investigate the effect of colchicines with duration time and LEDs treatment on plant regeneration of *Bulbophyllum auricomum*. Thazin orchid were treated with 2mg/l colchicines and MS liquid medium for 6, 12, 24 and 48 hours. And then, transfer to MS solid medium supplement with charcoal 2g/l, BAP 3mg/l and NAA 2mg/l and under LEDs treatment such as white, blue and red for plant regeneration. Among of them, the highest number of shoot regeneration and root regeneration was obtained from the colchicine treatments duration time 6, 12 and 24 hours with white LEDs treatment.

Key words: colchicine, LEDs

Introduction

Orchidaceae family has more than 1000 genera and approximately 22,000 species (Dodson, 2016). Orchids occur throughout the world, they are abundant in tropical regions of America, Africa and Asia, as these are the main regions where orchids are cultivated on large scale. Orchids are most remarkable due to their diversity and floral structure. Most of orchid varieties are epiphytic, they use rocks objects for support and derive their nutrients and water from the atmosphere and debris, however some species grow in the ground, under forest or grassland areas (Robert & Calaway, 1960). The genus *Bulbophyllum* belonging to the subfamily Epidendroideae, is the second largest genera of flowering plants with more than 2000 species (Smidt *et al.*, 2007, Govaerts *et al.*, 2017). These species spread throughout the tropics, and the subtropics, especially most of them are found in Asia. The pseudobulbs or thickened stem is a very useful storage organ for food and water and function like bulbs. *Bulbophyllum* species have many medicinal uses in Africa and Asia (Lawler, 1984). In China, some species are used to treat primary tuberculosis and stomach cancer (Pridgeon *et al.*, 2014). The pseudobulbs are used as a tonic for rejuvenation (Jonathan & Raju 2005, Teoh 2016). *Bulbophyllum auricomum* Lindl.,

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the royal flower of Myanmar, is an epiphytic and deciduous orchid. It is found in Myanmar, Thailand, Sumatra and Java. It flowers once a year and the blooms have an attractive perfume and has high horticultural value in local market.

Conventional propagation of *Bulbophyllum auricomum* by asexual method using pseudobulbs has become limited by habitat destruction and over-exploitation. *In vitro* propagation is one of the best conservation and rapid multiplication of the important endangered species. Colchicine treatment has become a common tool use for polyploid induction in many plants. The process can occur naturally or through human manipulation.

In general, polyploid plants exhibit superior phenotypes to those of diploids such as stronger stems, and thicker and larger leaves, flowers, fruits, and seeds. Polyploid induction can be used as a means to create and select new and better breeds for further use. In order to produce polyploidy plants, the chemical colchicine is widely used because of its effectiveness and availability but it is toxic to cells. Commercial micropropagation laboratories, light source are one of the most important factors controlling plant development. In this experiment, three light-emitting diodes (LEDs) are used such as White, Red and Blue. In the past decade, considerable progress has been made on the application of light-emitting diodes (LEDs) as a potential alternative light source for *in vitro* plant growth and morphogenesis. However, *Bulbophyllum auricomum* orchids are effect of different LEDs wavelength in the morphogenesis induction and growth of this species *in vitro* has not been evaluated. Therefore, in this study, the experiments was carried out to find the ways for efficient plant regeneration of *B. auricomum* through colchicine effect with duration time and LEDs treatment.

Materials and Methods

Source of plant material

Plantlet of Thazin orchids were obtained from Myanmar Floral and Biotech Co. Ltd. The plantlets were subculture on MS solid medium at pH 5.4.

Colchicine treatment and LEDs treatment

Plantlet were treated with 2mg/l colchicines and incubated on a rotary shaker for 6, 12, 24 and 48 h. After the end period of treatment, then transferred to MS medium supplemented with BAP 3 mg/l, NAA 2mg/land placed into different with LEDs treatment such as white, red and yellow. The survival rate, morphological,

physiological were recorded at the 21 days after the culture. All experiments are carried out in Chonbuk National University, Korea.

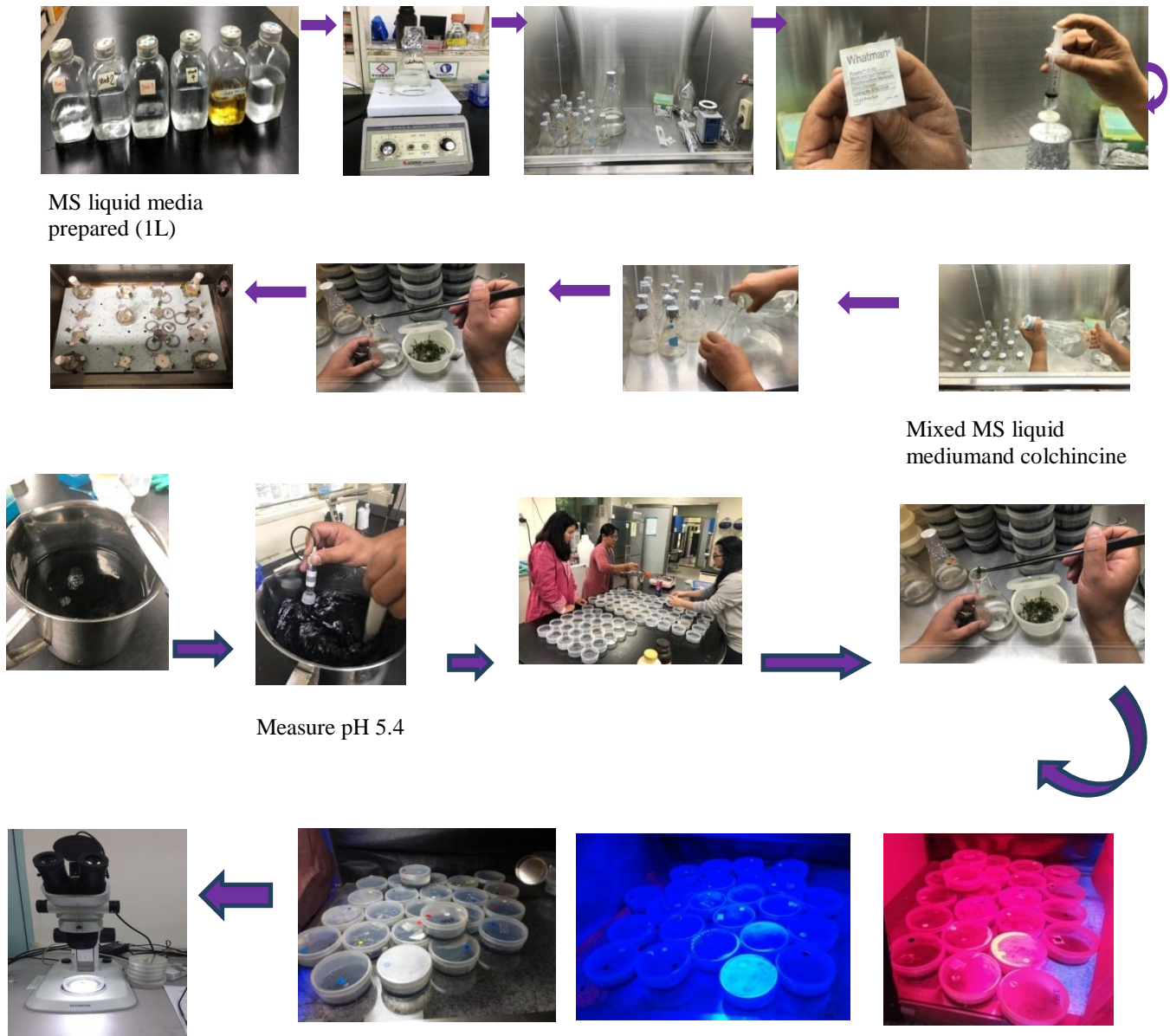


Figure (1) Procedure of Experiment

Result

Diagnostic characters of *Bulbophyllum auricomum* Lindl.

Bulbophyllum auricomum Lindl., the royal flower of Myanmar, is an endangered, epiphytic and deciduous orchid. Thin leaves that are often not present at blooming which is on an arching. The inflorescence consists of a many-flowered nodding raceme. Early winter and has fragrant flower.



Figure (1) Habit of *Bulbophyllum auricomum* Lindl

Table (1) Effect of colchicine solution and LED treatment of *Bulbophyllum auricomum* L.

LED treatment	Colchicine treatment time	Shoot regeneration	Root regeneration
White	6hours	none	present
	12hours	present	present
	24hours	present	present
	48hours	none	none
Red	6hours	none	present
	12hours	present	present
	24hours	none	none
	48hours	none	none
Blue	6hours	present	present
	12hours	present	none
	24hours	none	none
	48hours	none	none

This result showed after three weeks culture

	leave number	leave length(cm)	root number	root length(cm)
1	2	1.5	1	1.5
2	2	1	1	0.5
3	1	0.5	1	0.5
4	3	1.3	2	1

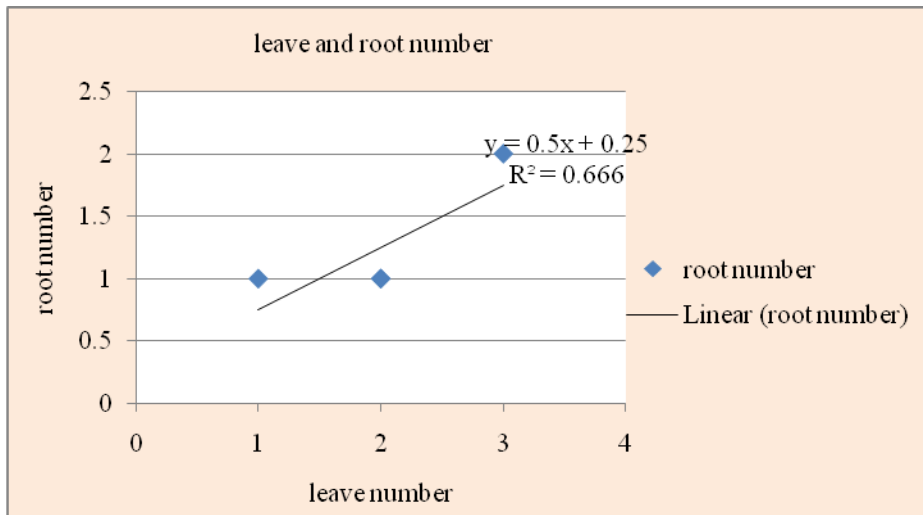


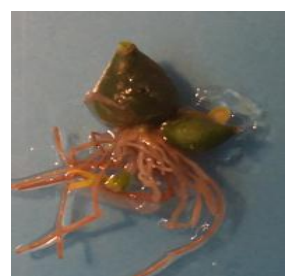
Figure (2) Correlation between leaf numbers and root numbers



12 hours



48 hours



6 hours

48 hours

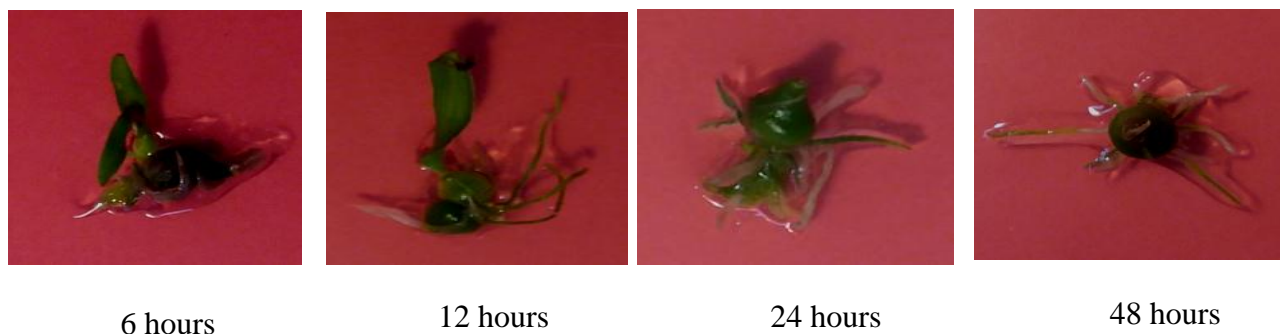


Figure (3) root and shoot regeneration in LEDs treatment

Effect of colchicine solution and LEDs treatments on % survival rate in *B. auricomum* Lindl.

LED treatment	Colchicine treatment time	Survival rate%
White	6hours	70
	12hours	90
	24hours	90
	48hours	50
Blue	6hours	70
	12hours	90
	24hours	60
	48hours	50
Red	6hours	80
	12hours	70
	24hours	60
	48hours	50

Discussion and Conclusion

As there are still a very few studies, it can be seen that *in vitro* regeneration of *Bulbophyllum auricomum* L. using tissue culture. In the present study, the different soaking duration in 2mg/l of colchicine for 6, 12, 24 and 48 hours had significant

influence on plantlet growth after three weeks of treatment. Lower duration (6h) and higher duration (48h) of colchicine showed 90% and 50% survivability respectively. The interaction between concentration and the duration of the colchicine treatments found that longer duration reduced survival of plantlet. Using longer duration, chlorophyll contents were decreased and plantlets were died after treated. (Thao *et al.*, 2003; Atichart and Bunnag, 2007). Similar results were also reported by Ngyuyeb *et al.*, 2003 and Pincherio *et al.*, 2000. For long time period, the survival rates were reduced to be zero because plantlets accumulate toxicity from colchicine which causing to cell destruction. The shoot proliferation and growth of *B. auricomum* L. were significantly affected by different light treatments *in vitro*. The result indicated a significant increase in the number of shoots and root per explants white LED. White light is the major source for the plant growth but certain plants needs its component light rays of variable wavelength for their growth and development. It is concluded that, the efficiency of polyploid induction, base on the *in vitro* application of colchicines to *B. auricomum* L., plantlets of this study will multiply and observation about plant growth, plant morphology such as the number of leaves and flower per plant, plant size and flower size and determine chlorophyll content for the further experiment. The use of a White LED sources was good at promoting the shoot proliferation and growth. White LED may be used for *in vitro* growth. The result demonstrated the effectiveness of a radiation system using LEDs for micro propagation.

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