EFFECTS OF BENZYL AMINO PURINE (BAP) DIFFERENT CONCENTATION FOR SHOOT MULTIPLICATION OF *NEPHENTES AMPULLARIA JACK*

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ABSTRACT

Nephentes ampullaria Jack is a carnivorous plant that can be found in Malaysia, Borneo, the Maluku Islands, New Guinea, Sumatra, and Thailand. City development and human activity had affected the natural habitat of this unique plants. Pitchers of N. ampullaria commonly used for "Lemang periuk kera" in Malaysia. Although, the status of N. ampullaria in the Red List is Least Concern (LC), day by day, the plant getting difficult to be found. Tissue culture is an alternative way to propagate and plant it back to its habitat as well as for the production of its pitchers for lemang production. FRIM has made proactive initiative to produced tissue culture plants of N. ampularia for conservation and for commercial production of this species. Shoot multiplication medium played an important role to ensure the growth of healthy new shoots produced. In this study, different concentration of Benzyl amino purine (BAP) ranging from (0, 0.5, 1.0, 2.5 and 5.0 mg/L) in MS basal media were used as plant growth regulator for shoot multiplication of N. ampullaria. Cotyledonary seedlings were cultured in these medium and incubated in culture room at a temperature of 22.0 ± 2.0°C. After 8 weeks of culture, all cultures were observed and data obtained were analysed using the Duncan's Multiple Range Test (DMRT). Results obtained showed that MS basal media added with 2.5 mg/L BAP is the optimal medium for shoot multiplication of N. ampularia with the highest mean number of new shoots (2.8 ± 0.26) compared to others.

Keywords: *Nephentes ampullaria, Tissue culture, shoot multiplication*

INTRODUCTION

Nephentes ampullaria belong to Nepenthaceae family and can be found in Malaysia, Borneo, the Maluku Islands, New Guinea, Sumatera and Thailand. *Nepenthes ampullaria* can be identified through its distinctive globular-urceolate pitchers with reflexed, linear-oblong lids (Cheek & Jebb 2001). The unique plant's pitchers produce a pitfall trap for invertebrate prey attracted to nectar secretions from the underside of pitcher lids, with 59 infaunal prey species recorded across its range (Lloyd 1942; Adlassnig et al. 2011).

People had been collecting *Nephentes sp.* plant including *Nephentes ampullaria* for its unique shape pitcher for its horticulture value. Other than that, in Malaysia, pitchers of *N. ampullaria* commonly used for "Lemang periuk kera". It usually served and prepared during festive season such as Hari Raya Aidifiltri and other celebration.

According to the RED List, the status of *N. ampullaria* was listed as Least Concern (LC). But due to human activities and space clearing for city development had disturbed the natural habitat of *N. ampullaria*. Improper harvesting method of N. *ampullaria* pitcher also can affect the population of this species. In order to make "lemang periuk kera", only the suitable pitcher should be harvested instead of the whole plant.

To restore the population of *Nephentes ampullaria* in its natural habitat, FRIM has made proactive initiative to produced tissue culture plants of *N. ampularia* for conservation as well as ~~and~~ for commercial production of this species. Propagation through tissue culture method will ensure the production of planting material without disturbing the existing colony and habitat. *In vitro* propagation will also be the fastest way to propagate this species as it is known to be a slow growing plant naturally.

This study aim to find the optimum Benzyl amino purine (BAP) concentration in MS basal medium for *Nephentes ampullaria in vitro* shoot multiplication and shoot generation.

MATERIAL AND METHODS

Plant material, surface sterilization and *in vitro* germination

~~I~~*Nephentes ampullaria* seeds were collected at Tanjung Malim, Perak. The ~~coated~~ seeds were surface sterilized using 50% Clorox® for 30 minutes and rinsed with sterile distilled water for 3 times. Seeds were then cultured in MS basal medium containing 0.5 mg/L BAP and incubated in culture room at a temperature of 22.0 ± 2.0°C for *in vitro* germination process.
Shoots Multiplication

In vitro germinated seedlings produced were used as stock explants for shoot multiplication experiments. The three-leaf seedlings were used as explants and sub-cultured into full strength Murashige & Skoog basal medium supplemented with different concentration of Benzyl amino purine (BAP) (0, 0.5, 1.0, 2.5 and 5.0 mg/L). The medium pH was adjusted to 5.8 before autoclaving. Five bottles of medium were prepared for each treatment, and each bottle contained three explants. The number of shoots induced per explants was observed after 8 weeks of culture.

RESULT AND DISCUSSION

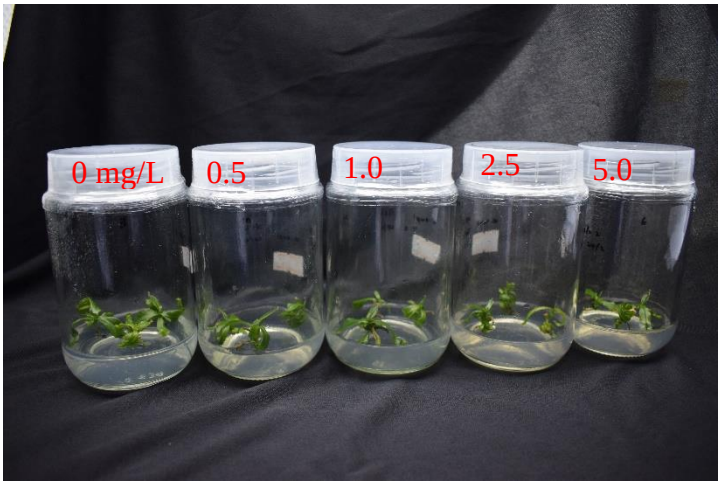
Shoot multiplication *of Nephentes ampullaria* were observed after 8 weeks of culture. It was observed that there was significance difference between MS basal medium without and added with BAP. Among the five treatments, it was observed that MS basal medium with 2.5 mg/L BAP produced the highest mean shoot numbers (2.8 ± 0.26) followed by MS basal medium with 5.0 mg/L BAP (2.73 ± 0.18) and MS basal medium with 1.0 mg/L BAP (1.47 ± 0.27). Meanwhile MS basal media without an addition of BAP only produced 0.2 ± 0.11 mean number of shoots. Our results showed that shoots were increased directly proportional with the increment of BAP concentration from 0 mg/L BAP to 2.5 mg/L BAP but decreased after that with 5.0 mg/L BAP. Budisantoso et al (2018) also reported that the addition of BAP to a certain concentration in ½ MS promote the development of *N. ampullaria* new bud but after the optimum concentration it will showed the negative effects. The optimum medium for shoot multiplication and shoot regeneration in our study is MS basal medium containing 2.5 mg/L BAP.

Table 1: Effect of different BAP concentration on *N. ampullaria* shoot multiplication and leaf length

Treatment (BAP)	Mean shoots bud	Longest leaf length (cm)
MS + 0.0 mg/L	0.20 ± 0.11$_c$	1.5
MS + 0.5 mg/L	1.07 ± 0.23$_b$	2.2
MS + 1.0 mg/L	1.47 ± 0.27$_b$	2.5
MS + 2.5 mg/L	2.80 ± 0.26$_a$	1.5
MS + 5.0 mg/L	2.73 ± 0.18$_a$	1.5

Cytokinins such as BAP can influence various traits of plant growth, development and physiology such as seed germination, apical dominance, flower and fruit development, leaf senescence and plant-pathogen-interactions etc (Akhtar et.al. 2020).

Figure 1: *N. ampullaria* in MS basal media with different concentration of BAP after 8 weeks of culture



For the length of longest leaf, tt was observed that MS basal media supplemented with 1.0 mg/L BAP had a very significant effect followed by added with 0.5 mg/L. There were no significance different in term of longest leaf for the other three treatments. These observation were also supported by Budisantoso *et al*. (2018) that the higher the BAP concentration, the length of the leaf will increase when compared with the control, then at a certain concentration it will decrease. As to get a larger plant for acclimatization, we need to sub-culture the explants into MS basal medium with 1.0 mg/L BAP. Large and healthy plants will have higher survival rate during acclimatization process compared to small one.

CONCLUSION

Result obtained proved that *N. ampullaria* can be multiplied and propagated using *in vitro* propagation technique. Tissue culture can fasten the production of planting material that will help to restock *N.ampullaria* in its natural habitat. *N. ampularia* can be planted to cater the demand of its pot for "Lemang Periuk Kera".

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