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## **Effect of Medium Strength and Hormones Concentration on Regeneration of *Pogostemon cablin* using Nodes Explant**

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### **ABSTRACT**

*Pogostemon cablin* also known as Patchouli belongs to the family Lamiaceae is an important aromatic plant producing patchouli oil from its leaves extraction. The main objective of this study was to develop an effective method for *in vitro* propagation of Malaysia cultivated *P. cablin*. The effect of varying the strength of solid MS medium was investigated using nodes explants. Different concentrations of 6-Benzlamino purine, BAP (0, 0.25, 0.5, 1.0, 2.0 mg L<sup>-1</sup>) and alpha naphthalene acetic acid, NAA (0, 0.25, 0.5, 1.0, 2.0 mg L<sup>-1</sup>) were added to both full and half strength MS (Murashige and Skoog) medium for regeneration of this plant. After 4 weeks, the shoots regeneration from the nodal segments was obtained in all medium. Then, clumps of shoots (5 shoots) were separated and transferred to five MS medium concentrations (0.25 mg L<sup>-1</sup> BAP, 0.5 mg L<sup>-1</sup> BAP, 0.25 BAP/0.25 NAA and 1.0 BAP/0.25 NAA). MS supplemented with 0.25 mg L<sup>-1</sup> BAP alone gave the highest number of shoots (32.93±3.93) and length (3.80±0.27 cm) followed by MS supplemented with combination of 1.0 mg L<sup>-1</sup> BAP and 0.25 mg L<sup>-1</sup> NAA hormones. There was also a significant effect of hormones and media strength on the number of shoots obtained. The statistical analysis done showed that there was very high interaction between both factors of hormones concentration as well as the medium strength. In the other words, the number of shoots obtained to hormones concentration depends on medium strength and vice-versa. In conclusion, *in vitro* regeneration of *P. cablin* from node explants in MS medium supplemented with BAP or NAA was successfully obtained. The healthy propagules that were obtained in this research can be used for further procedure of acclimatization.

**Key words:** Patchouli, *in vitro* propagation, shoots regeneration, *pogostemon cablin*, full strength medium, half strength medium, plant growth regulators

### **INTRODUCTION**

*Pogostemon cablin* or patchouli famously known by its commercial name nilam is an aromatic plant. It is also the main source of patchouli oil obtained in their essential oil through extraction process. It has benefits perfumery industry for many years. Patchouli oil has fixative properties which make the smell last longer on the skin (Kongkathip *et al.*, 2009; Sugimura *et al.*, 2005). Since this substance need high market demand, their supply has become a major concern in producing continuous supply to the industry. It has been claimed that patchouli has

non-synchronous flowering system which that the plant never flowers (Sjamsudin *et al.*, 1997). As a result, conventional vegetative method by stem cutting (Swamy *et al.*, 2010) has been alternatively practiced by the planters but it only result in limited stock for this plant. It is due to the presence of potential diseases result from viral infection, fungal and also bacterial infection as well as insect pests (Misra, 1996). These constraints lead a bad impact to the industry. It is important to develop a reliable protocol of regeneration system suitable for particular cultivars such as in *Bacopa monnieri* to serve its rising market demands (Soundararajan and Karrunakaran, 2011). As, an alternative method of propagating of the plant, plant tissue culture technique can gave advantage in multiplying million folds of a desired plant (Warar *et al.*, 2008).

Study on *in vitro* propagation of *P. cablin* using leaf explants was extensively done in previous study with the use of different kinds of auxin and cytokinin in different range of concentrations. In many literature reports of propagating plants via tissue culture, the best selection of explants played major roles in obtaining efficient shoots regeneration such as *Swertia chirata* (Jha *et al.*, 2011) and *Capsicum annum* L. (Otroshy *et al.*, 2011).

Thus, this study was done to regenerate shoots using nodes segment explants of *P. cablin* in both half and full strength MS medium with different concentrations of BAP and NAA. Nodes segment is a part of stem in which new growth can occur naturally. It may produce new leaves, flowers and cones and even stems itself thus made it a perfect explants to be used for growing new desired plants. Plant growth regulators that were implemented into MS basal medium also play a vital role in shoots and roots formation (Nasir Uddin, 2006) and also the seed yield (Tiwari *et al.*, 2011).

## MATERIALS AND METHODS

This research was conducted in Plant Tissue Culture Laboratory from December 2010 to May 2011.

**Sample collection and sterilization:** Patchouli or nilam was collected from Ayer Keroh, Melaka. The plants descriptions, natural habitat, growth, distribution, scientific studies and uses were documented. Voucher specimen was deposited at the Herbarium Institute of Bioscience, University Putra Malaysia. In the laboratory, plants' parts were cleaned and sterilized using bleach (Clorox 20%) for 15 min prior to culture. Aseptic techniques were applied to each step in the laboratory.

**Plant regeneration:** Nodal segments explants were cultured on full and half strength MS (Murashige and Skoog) medium. The explants were placed on MS media with different concentration of NAA hormone (0, 0.25, 0.5, 1.0, 2.0 mg L<sup>-1</sup>) and BAP hormone (0, 0.25, 0.5, 1.0, 2.0 mg L<sup>-1</sup>). The culture bottles were incubated in growth room at adjusted temperature of 25°C and under initial photoperiod of 16 h light and 8 h dark period. The number of shoots and percentage of regeneration were observed and recorded.

**Subculture:** After 4 weeks of initiation, the explants were subculture into fresh medium to ensure that the nutrients were enough to eliminate cell growth exhaustion. The clumps of shoots were separated and cultured into those five best concentrations which are 0.25 mg L<sup>-1</sup> BAP, 0.5 mg L<sup>-1</sup>

BAP, 0.25 mg L<sup>-1</sup> BAP/0.25 mg L<sup>-1</sup> NAA, 0.5 mg L<sup>-1</sup> BAP/0.25 mg L<sup>-1</sup> NAA and 1.0 mg L<sup>-1</sup> BAP/0.25 mg L<sup>-1</sup> NAA. Observation and measurement on plant regeneration, number and length of shoots were done and all the data been recorded.

**Statistical analysis:** The results were expressed as mean Value±Standard error mean. Two-way Analysis of Variance (ANOVA) at 95% confidence interval of probability was used in data analysis using Microsoft Excel 2007.

**RESULTS AND DISCUSSION**

Plant growth was monitored by the measurement on number of shoots in each explants as well as their percentage of regeneration. There were 25 concentrations of hormone involving BAP and NAA alone and in combinations. In literature reported, the hormones used were kinetin and IAA (indole-acetic acid) in various concentrations ranging from 0.5-2.0 mg L<sup>-1</sup> to obtain mass multiplication of three different genotypes of *Pogostemon cablin* marked as POG002, POG014 and POG021 (Santos *et al.*, 2010). Maximum number of shoots (175) was recorded in POG021 on MS media supplemented with 1.0 mg L<sup>-1</sup> kinetin and 0.5 mg L<sup>-1</sup> IAA using leaf explants.

Both full strength and half strength MS medium were tested. This experiment was tested using Complete Randomized Design (CRD) in which there were three replicates of five explants (25×2×3×5) in each concentration for both strengths.

Figure 1 shows the growth patterns of *P. cablin* nodes segment in full and half-strength of MS medium. Generally, the explants were succeeded to survive in most of the concentrations of

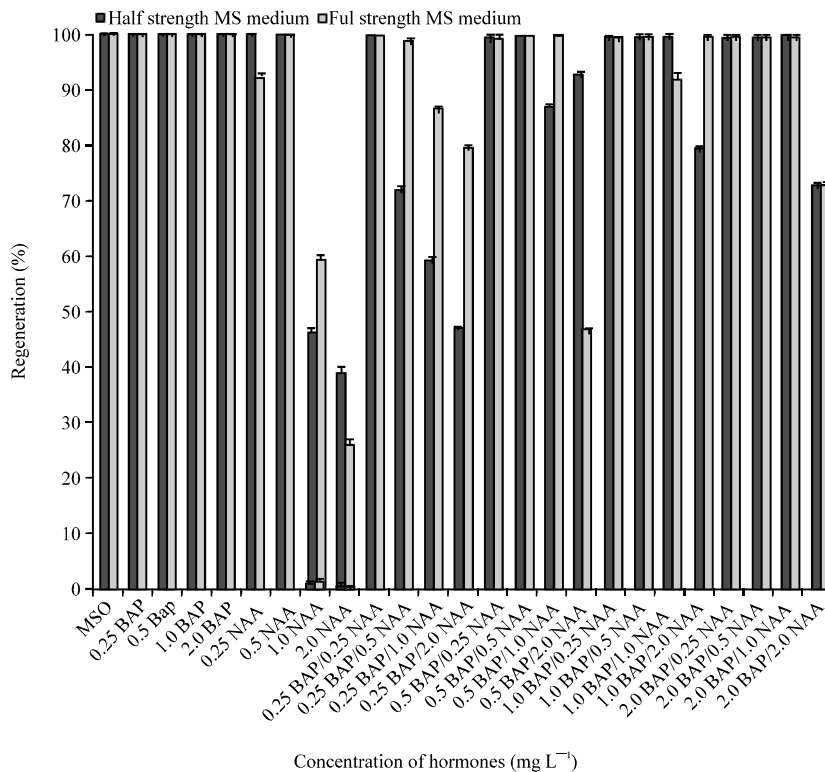


Fig. 1: The percentage of regeneration by nodes segments culture onto half strength and full strength MS medium supplemented with BAP and NAA hormones after 4 weeks culture

hormones supplemented in both strengths of MS medium. The percentage of regeneration was taken in 4 weeks after initiation of the nodes segments. Hormone BAP gave optimum regeneration (100%) in most of the cultures whereas; the percentage of regeneration was lower in the medium supplemented only with NAA and or with high level of concentration of BAP and NAA. The explants in lower percentage of regeneration exhibit slower growth rate which need longer time to produce many shoots.

In this study, the node explants showed responses to shoot formation within the first week of initiation. The node segments swelled and curled at the cut-edge followed by bud and shoots formation within a week more and ahead. Similar respond using leaf explants has also been observed in the study done by Paul *et al.* (2010).

Since the *P. cablin* cultures obtained in clumps after 4 weeks of initiation, the experiment was tested further in five selected concentrations (0.25 mg L<sup>-1</sup> BAP, 0.5 mg L<sup>-1</sup> BAP, 0.25 mg L<sup>-1</sup> BAP/0.25 mg L<sup>-1</sup> NAA, 0.5 mg L<sup>-1</sup> BAP/0.25 mg L<sup>-1</sup> NAA, 1.0 mg L<sup>-1</sup> BAP/0.25 mg L<sup>-1</sup> NAA) that gave highest number of shoots. The clump of shoots (5 shoots) were separated and subcultured into the fresh MS medium containing the five selected concentrations.

Based on Fig. 2, a significant number of shoots were produced in full strength of MS medium within 4 weeks after initiation. In overall treatments in half strength medium, it was recorded that the number of shoots obtained was lesser compared to full strength medium. The finding was supported by Kumar *et al.* (2006) who stated that the *Litchi chinensis* plant gave better frequency

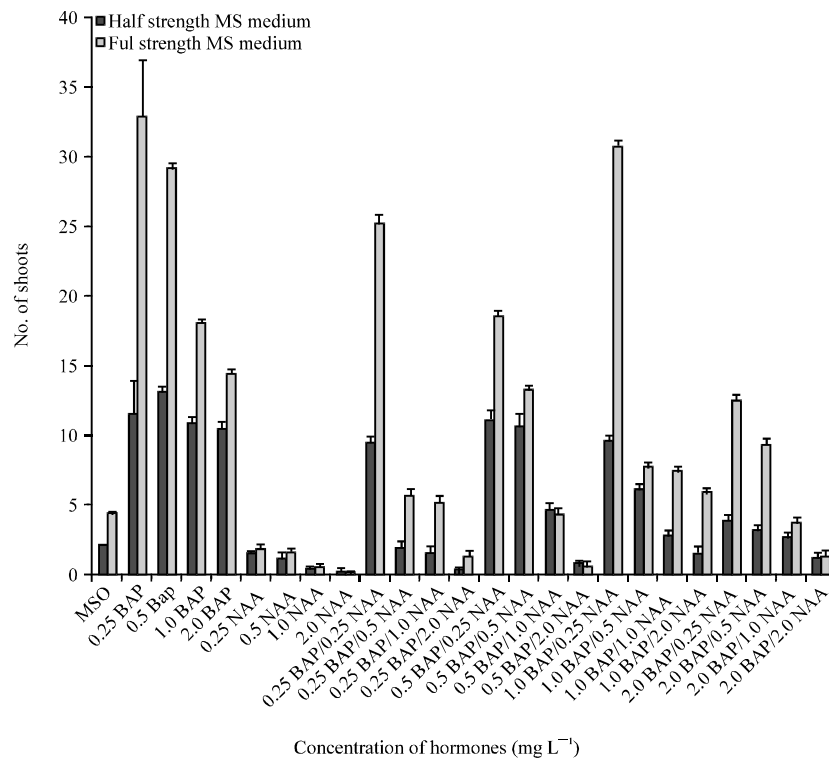


Fig. 2: The number of shoots regenerated in different concentrations of hormones supplemented in MS medium both in full and half strength after 4 weeks culture

of shoots in full strength medium that enriched with different types of plant growth regulators. Two-way ANOVA analysis showed that the strength of a medium was highly affecting the number of shoots formation with P value was less than 0.0001 ( $p < 0.0001$ ). In contrary, both full and half strength of MS media gave significant number of leaves of ginger, *Zingiber officinale* Rosc. (Villamor, 2010). However, the number of shoots were comparable in both full and half strength and it can be improvise by the addition of KNO<sub>3</sub> (Villamor, 2010). Addition of hormones in the MS media was proved to be highly significant as  $p =$  value was less than 0.0001 ( $p = 4.0107E-92$ ). Shoot and root induction requires the addition both auxin and cytokinin supplemented in MS medium (Nasir Uddin, 2006). According to the result obtained in this study, the highest number of shoots per explants ( $32.93 \pm 3.93$ ) was obtained from MS full strength media supplemented with  $0.25 \text{ mg L}^{-1}$  BAP followed by combination of  $1.0 \text{ mg L}^{-1}$  BAP and  $0.25 \text{ mg L}^{-1}$  NAA ( $30.7 \pm 0.5$  shoots per explant). However, there was no effect on shoot differentiation in MS medium supplemented with NAA. This finding is similar to the study done by Misra (1996) which proved that the presence of auxin alone will not give any observation in the formation of shoots within 4 weeks. Statistical analysis using ANOVA gave P value less than 0.0001 ( $p = 2.2180E-57$ ) indicates that the interaction between hormones and the strength of media was also highly significant to the number of shoots produced per explants.

Increasing level of BAP concentrations showed declining in number of shoots. The elevation of BAP level contributed to the declining in the formation of multiple shoots thus reduces the number of shoots obtained (Paul *et al.*, 2010). On the other hand, plant growth regulator cytokinin is vital for shoot regeneration using leaf explants (Paul *et al.*, 2010). In this case the use of low concentration of cytokinin ( $0.25 \text{ mg L}^{-1}$  BAP) gave highest number of shoots number. The medium that produced maximum number of shoots also gave the highest length of shoots ( $3.80 \pm 0.27 \text{ cm}$ ). This proved that low level of BAP is sufficient to obtain the optimal shoots number of *P. cablin* using node explants. Venkatramalingam and Ebbie (2011) also stated that rising concentrations of auxin and cytokinin will suppressed the frequency of shoots regenerated.

According to Paul *et al.* (2010), high concentrations of BAP combined with low concentration of NAA tested on leaf explants resulted in the formation of multiple shoots. The addition of cytokinin in MS medium to auxin supposedly improves the number of shoots (Siddique and Bari, 2010). The result shown differently in this study as the highest shoot formation was recorded in MS medium supplemented with BAP alone. Based on the result, MS medium supplemented with  $1.0 \text{ mg L}^{-1}$  BAP and  $0.25 \text{ mg L}^{-1}$  NAA resulted in considerable number of multiple shoots formation tested on nodes segment explants. However, as shown in Fig. 3, the length recorded for this treatment ( $1.96 \pm 0.62 \text{ cm}$ ) do not gave advantage against treatment of low concentration of BAP alone ( $0.25 \text{ mg L}^{-1}$  BAP) which is  $3.80 \pm 0.27 \text{ cm}$ .

It was found that the same concentrations of cytokinin and auxin ( $0.25 \text{ mg L}^{-1}$  BAP/ $0.25 \text{ mg L}^{-1}$  NAA) and high concentration of cytokinin ( $1.0 \text{ mg L}^{-1}$  BAP/ $+0.25 \text{ mg L}^{-1}$  NAA) caused the formation of callus. As a result, the frequency of multiple shoots formation was reduced which decrease the number of shoots obtained. Siddique *et al.* (2006) also stated that callus production was controlled by hormones in terms of its concentration as well as the presence of both auxin and cytokinin in the MS medium.

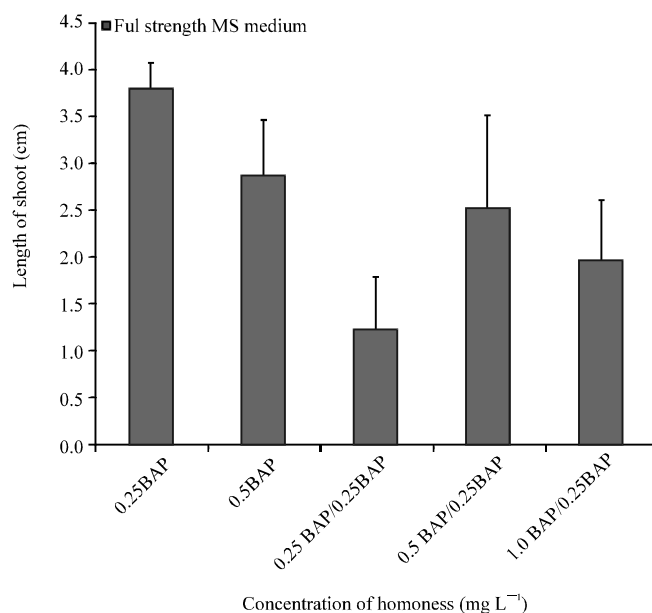


Fig. 3: The length of shoots regenerated in five best concentrations of hormones supplemented in full strength MS medium after 4 weeks culture

## CONCLUSION

In conclusion, an efficient method was established for direct shoot regeneration from the nodes segments explants of *P. cablin* on full strength MS media. A cost effective method of propagation through tissue culture technique was successfully achieved which could be use to overcome the constraints and fulfill the market demand. The healthy propagules that were obtained in this research can be used for further procedure of acclimatization.

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