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Improvement of Shoot Regeneration of Potentially Medicinal Plant *Melaleuca alternifolia* Via Axillary Shoot

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Graphical abstract

Abstract

The aim of this study is to establish an improved shoot regeneration of potential medicinal plants, *Melaleuca alternifolia*. The essential oil of these plants is useful in the cosmetic and pharmaceutical industries. Despite the importance of this species, *in vitro* regeneration was limited. Therefore, the present study use various concentrations of plant growth regulators to improve shoot regeneration of these plants. The present study shows that axillary shoot gave the best response to the treatment containing 0.5mg/L BAP and 0.1mg/L NAA. The average number of shoots is 23 with 77% of shooting. For rooting experiment, 35% of axillary shoot were rooted when cultured on MS medium supplemented with 1.5mg/L IBA. *In vitro* plantlet was later developed after 3 months.

Keywords: *Melaleuca alternifolia*; tea tree; plant growth regulators; shoot; root, axillary shoot; medicinal plants

Abstrak

Kajian ini bertujuan untuk menentukan dan mempertingkatkan regenerasi pucuk tumbuhan ubatan, *Melaleuca alternifolia*. Minyak pati tumbuhan ini berguna dalam industri kosmetik dan farmaseutikal. Walau bagaimanapun, regenerasi *in vitro* tumbuhan ini sangat terhad di sebalik kepentingannya. Oleh itu, kajian ini menekankan penggunaan pelbagai jenis hormon tumbuhan dalam merangsang dan mempertingkatkan pengeluaran pucuk. Kajian mendapati bahawa pucuk aksilari menunjukkan tindak balas optimum terhadap media MS yang mengandungi 0.5mg/L BAP dan 0.1mg/L NAA. Purata bilangan pucuk yang terhasil adalah 23 dengan 77%. Sebanyak 35% daripada pucuk aksilari yang tumbuh didapati mengeluarkan akar apabila dikultur di atas media MS yang mengandungi 1.5mg/L IBA. Selepas 3 bulan, tumbuhan *in vitro* dihasilkan.

Kata kunci: *Melaleuca alternifolia*; pokok the; hormon tumbuhan; pucuk aksilari; akar, tumbuhan ubatan

1.0 INTRODUCTION

Melaleuca alternifolia is tea tree belongs to Myrtaceae family and native at New South Wales, Australia. It grows wildly in swampy, low lying wetlands (Homer *et al.*, 2000) or near rivers (Riedl, 1997). Tea tree oil is used as a natural remedy for antibacterial and antifungal for the infections of skin and mucosa (Kiong *et al.*, 2007). It is used as an antiseptic agent and anti-inflammatory which has commercial value in cosmetic industry and toiletries. Unfortunately *Melaleuca* species, like most forest trees, have long generation time and often display changes in growth rate and morphology with time. Therefore, micro propagation through tissue culture offers more advantages for the establishment of fields' propagation. Until now, however only few literatures have been reported regarding their *in vitro* propagation. De Oliviera *et al.*, (2010) reported high efficient of shoot proliferation via axillary shoot while bud proliferation from nodal segment by List *et al.*, (1996). De Oliviera *et al.*, (2010) found the use of both solid and liquid MS media containing 0.55µM and 1.11 µM BA respectively proved to be the best media in promoting shoot. High shoot number was also observed when nodal bud was used in the present of 4.5 µM BA in the liquid media as reported by List *et al* (1996). The addition of auxin was not critical in promoting root system, however addition of charcoal reduced rooting rate (De Oliviera *et al.*, 2010). 80% of nodal bud was rooted when MS medium supplement either with 0.1µM or 1 µM IAA was used as reported by List *et al.*, (1996). Therefore, the present study aimed to improve shoot regeneration and establish protocol for *Melaleuca artenifolia* regeneration using solidified MS media.

2.0 MATERIALS AND METHODS

Mature plant of *Melaleuca alternifolia* was obtained from Sime Darby, Malaysia. For sterilization, the axillary shoot were carefully excised and rinsed under running water for 15 minutes followed by immersion in 70 % (v/v) ethanol for 30 seconds. The explants were then disinfected with 10% (v/v) sodium hypochlorite containing 2 drops of Tween 20 for 10 minutes, and several times rinses in sterile distilled water. The sterilized explants were then blotted dry on sterile filter paper before being cultured on the MS (Murashige & Skoog, 1962) culture medium solidified with agar. Various plant growth regulators were used such as 0.5mg/L BA and a combination of 0.5mg/L BA and 0.05, 0.1m/L NAA. For rooting purposes IBA and NAA at concentration ranging from 0.5mg/L to 1.5mg/L were used. All plates were incubated at 27°C day/night temperatures with a 16 hour photoperiod and irradiance of 70µmol $m^{-2}s^{-1}$. At the end of the study, data such as shooting percentage, number of shoot per explants and rooting percentage were collected. Each experiment had 3 replicates and was repeated 2 times.

3.0 RESULTS AND DISCUSSIONS

In this study, MS medium containing a combination of 0.5 mg/l BA and 0.1 mg/l NAA showed the highest shooting percentage. As shown in Figure 1, 77% of shoots were induced from axillary shoot explants with a number of 23 shoots per explants on MS media containing 0.5 mg/L BA and 0.1 mg/L NAA. Other treatments were also producing shoot but with lower percentage. The percentage of shoot formation was ranged from 20% to 50% when axillary shoot was cultured with other treatments. The lowest shoot percentage was observed on MS media supplemented with 0.5 mg/L BA and 0.005 mg/L NAA at 3%. However, the control treatment also produced shoot at 10% but with slower rate, within 4 weeks compared to other treatment within 2 weeks. After 2 weeks in culture, the shoot formed was 0.3 – 4.0 mm length and greenish in color and cluster of shoot was formed later at 6 weeks.

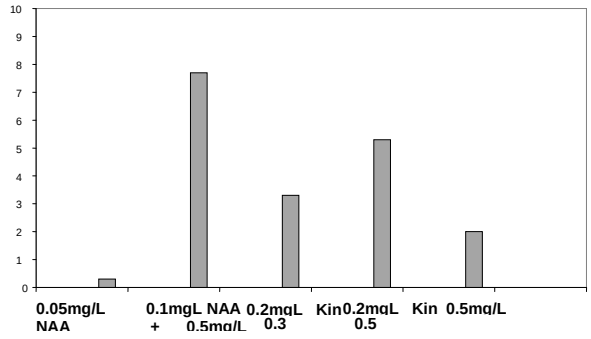

Figure 1 Percentage of shoot induction when different concentration of plant growth regulators were used after 4 weeks in culture

Rooting was observed at 35% when the axillary shoot was cultured on MS medium supplemented with 1 mg/L IBA followed by 1.5 mg/L IBA at 33%. No rooting was observed on auxin free medium (Figure 2). The axillary shoot explants were sub cultured on MS medium containing 0.5 mg/l BA and 0.1 mg/l NAA for further 6 weeks and became *in vitro* plantlet after 3 months (Figure 3).

De Oliveira *et al.*, (2010) have reported that optimal multiple shoots appeared from axillary shoot in *Melaleuca artenifolia* were only obtained when MS media was used. In agreement with this, the present study showed that solidified MS media with high BAP concentration have a potential to induce shoot at high percentage as average of shoot number was 23 compared to 5.5 when solid MS medium was used. They also found that the use of liquid MS increase the shoot multiplication up to 11.8 shoot number because of nutrient and BA diffusion and absorption are easier compare to solid medium. In our study indicated that the highest shoot number was also observed when MS solidified medium was used containing 0.5mg/L BA and 0.1 mg/L NAA compared to 5.6 shoot number on 0.55µM BA (De Oliveira *et al.*, 2010). This may be because of the differences in terms of their variety. The use of BA in inducing shoot was also reported by List *et al.* (1996) where 5.5 shoots per segment was observed from nodal segments were cultured on MS solidified medium supplemented with 4.5 µM BA at the end of 9 week of culture. Others also reported that varying degree of shoot multiplication when using MS media in *Acacia* sp and *Anacardium occidentale* (Vengadesan *et al*., 2002; Mneney & Mantell, 2002).

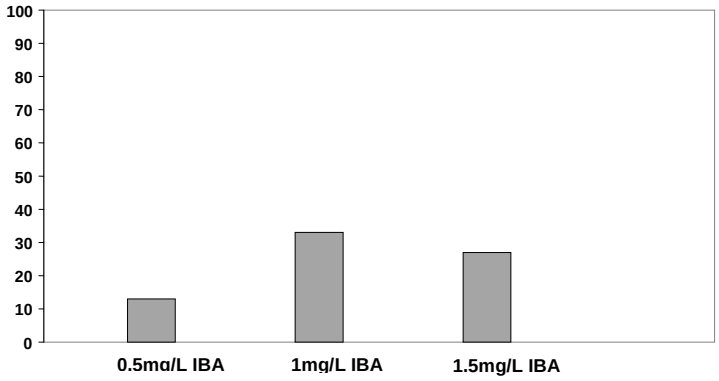

Figure 2 Percentage of rooting when explants were cultured on MS medium supplemented with different concentration of IBA after 6 weeks in culture

Using axillary shoot as an explants, 33% rooting was achieved in MS medium supplemented with 1mg/L IBA however no rooting was observed in media without hormone. The requirement of low concentrations of auxin or free auxin medium has been reported in inducing root in other Eucalyptus species (Ito *et al.*, 1996) while rooting was achieved in *M. alternifolia* when using IAA, IBA and NAA (List *et al.*, 1996; de Oliviera *et al.*, 2010). Similar results were also observed in the present study but with lower percentages. This observation could be due to low concentration of auxin used in the present study. Since the rooting performance was low, further study need to be conducted. Varying degree of response in rooting was also reported such as inclusion of sucrose in *Asparagus racemosus* (Bopana & Saxena 2008), *Passiflora edulis* (Isutsa 2004) and the strength of MS medium in *M. alternifolia* (de Olieviera *et al.*, 2010). The relation between auxins, carbohydrate type and rooting is complex and has not yet been elucidated. Therefore, future studies need to be explored on the establishment of acclimatization process since no field-established plants is presented in this study.

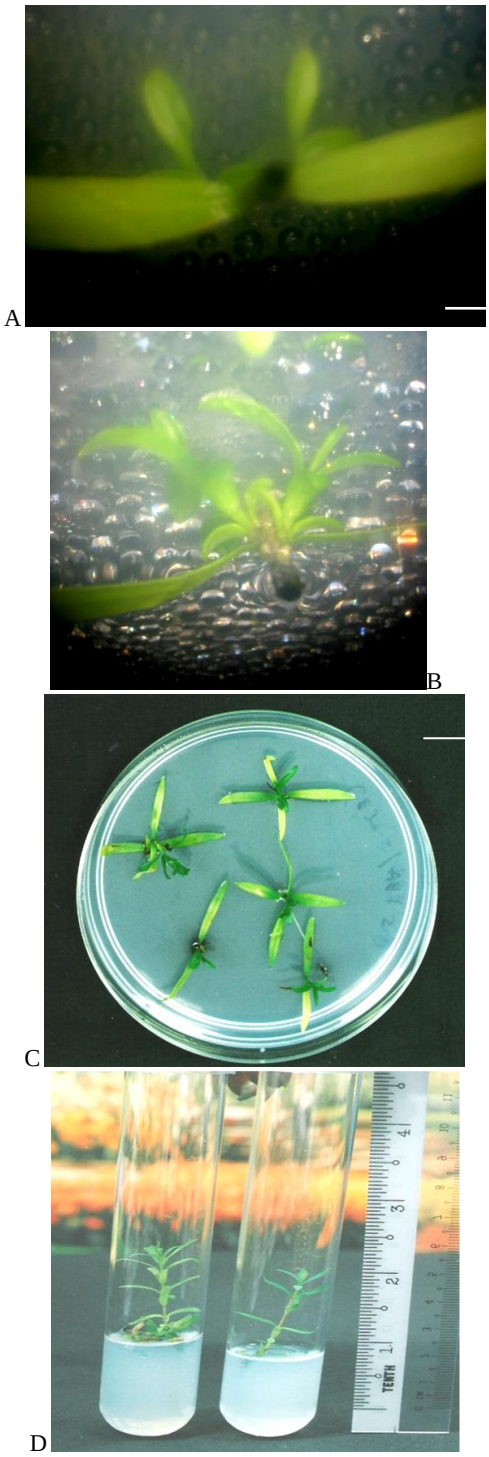

Figure 3 Regeneration of *Melaleuca artenifolia* via axillary shoots. (A) 3 weeks shoot derived from axillary (B) Shoot cluster after 6 weeks in culture. (C) Rooting on MS medium containing 1mg/L IBA. (D) In vitro plantlet achieved from axillary shoot after 3 months in culture. Bar= 1cm

4.0 CONCLUSIONS

In this study, shoot induction of tea tree was established using 0.5 mg/L BA and 0.1 mg/L NAA when using axillary shoot as explants. Rooting was observed at 33% when axillary shoot was cultured on MS media containing 1.0 mg/l IBA while no roots were induced in control treatment. Establishing the protocols in the present study will be used as a platform for further improvement of *in vitro* propagation of *Melaleuca alternifolia*. In addition, large scale propagation of these plants helps this valuable medicinal species from becoming endangered.

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