



Original Article

Micropropagation and callus induction of *Lantana camara* L. – A medicinal plant

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ABSTRACT

Lantana camara L., a medicinal plant, exhibits antimicrobial, fungicidal, insecticidal and nematocidal properties. Effective *in vitro* micropropagation and callus induction aid plant material production for bioactive compound studies and plant resource conservation. Shoot multiplication, root induction and callus formation were investigated. The results indicated a high shoot multiplication rate on Murashige and Skoog (MS) medium supplemented with 16.0 $\mu\text{mol/L}$ N6-benzyladenine (BA). Shoots showed good root regeneration with healthy plantlets on MS medium supplemented with 7.5 $\mu\text{mol/L}$ indole-3-butyric acid. A large callus size occurred with roots at the basal end of shoots on the media added with 20.0 $\mu\text{mol/L}$ or 40.0 $\mu\text{mol/L}$ of 1-naphthalene acetic acid (NAA) combined with 40.0 $\mu\text{mol/L}$ BA. Leaf explants were more suitable for callus induction. Media containing 0.0–40.0 $\mu\text{mol/L}$ BA in combination with 20.0 $\mu\text{mol/L}$ or 40.0 $\mu\text{mol/L}$ NAA could efficiently induce callus formation with the same level of relative growth rate. These plants and callus will be suitable sources of plant material for further study in producing bioactive compounds.

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Introduction

Lantana camara is a well-known medicinal plant of the family Verbenaceae which is mainly used as a traditional medicine and also as firewood and mulch (Kalita et al., 2012). Leaf extracts of *lantana* exhibit antimicrobial, fungicidal, insecticidal and nematocidal properties (Kalita et al., 2012; Reddy, 2013). Verbascoside, which possesses antimicrobial, immunosuppressive and anti-tumour activities, has been extracted from *lantana* (Kalita et al., 2012). Recently, research has emphasized feasible uses of *L. camara* in modern medicine (Srivastava et al., 2011; Kalita et al., 2012; Reddy, 2013).

Much research has revealed the success of *in vitro* culture for many plant species in the Verbenaceae (Steephen et al., 2010; Ravinder Singh et al., 2011; Srinivasan et al., 2012; Waoo et al., 2013). Several concentrations of plant growth regulators have been applied in *in vitro* culture techniques for many species (Balaraju et al., 2008; Steephen et al., 2010; Ravinder Singh et al., 2011; Srinivasan et al., 2012). Inducing bud break and shoot regeneration was found effective using 2.0 mg/L N6-benzyladenine

(BA) for *Vitex agnus-castus* (Balaraju et al., 2008) and 1.0 mg/L BA for *Vitex negundo* L. (Steephen et al., 2010). A concentration of 3.0 mg/L BA showed the most promising shoot multiplication effect for *Premna serratifolia* L. (Ravinder Singh et al., 2011) and *Tectona grandis* L.f. (Srinivasan et al., 2012). A range of BA concentrations from 0.1 to 0.7 mg/L induced the highest shoot number (three shoots per explant) in *L. camara* (Waoo et al., 2013). The *in vitro* regenerated shoots of *V. agnus-castus* later produced roots when transferred onto half-strength Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 0.1 mg/L indole-3-butyric acid (IBA) (Balaraju et al., 2008). A rooted shoot of *P. serratifolia* L. was achieved after nodal cutting using half-strength MS medium supplemented with 1.0 mg/L 1-naphthalene acetic acid (NAA) (Ravinder Singh et al., 2011). An important factor reported in several species with regard to callus induction is the type of explant, for example: shoot buds of *P. serratifolia* L. (Ravinder Singh et al., 2011), internodal segments of *T. grandis* L.f. (Widiyanto et al., 2005) and young leaves and shoot tips of *L. camara* (Saxena et al., 2013). In addition, there was variation in the plant growth regulators used to induce callus: only indoleacetic acid (IAA) (Ravinder Singh et al., 2011), BA (Srinivasan et al., 2012) and 2,4-dichlorophenoxyacetic acid (2,4-D) (Saxena et al., 2013) as well as a combination of BA and NAA (Widiyanto et al., 2005).

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A plant cell culture technique has been reported to supply a continuous and reliable source of natural products (Vijaya et al., 2010). In addition, the callus culture has also been reported to effectively produce some active ingredients and specific medicinal compounds equivalent or superior to that of intact plants, for example: flavonoid from *Centella asiatica* (L.) Urban, camptothecin from *Nothapodytes foetida*, catharanthine from *Catharanthus roseus* and anthraquinone from *Cassia acutifolia* (Tan et al., 2010; Vijaya et al., 2010; Hussain et al., 2012). In *L. camara*, three pentacyclic triterpenoids consisting of betulinic acid, oleanolic acid and ursolic acid were found to accumulate in callus growing on leaf disc explants. Their important activities were effective in killing cancerous cells (Srivastava et al., 2010). Additionally, the callus extract containing allelopathic compounds showed toxicity to the growth of *Salvinia molesta* Mitchell (Saxena et al., 2013).

Not only is the tissue culture technique beneficial in the production of bioactive compounds from plant material *in vitro*, but it also is beneficial for *ex situ* conservation. Using *in vitro* plant material can help to reduce the invasion of forests and the destruction of natural plants to obtain natural material. However, *in vitro* propagation of *L. camara* has been rarely reported. In the present study, an efficient technique for the micropropagation and callus induction of *L. camara* was developed. This technique will be valuable to produce plants and callus of *L. camara*, which can be used as sources for bioactive ingredient extraction.

Materials and methods

Explant preparation

A single shoot of *L. camara* was surface-sterilized by soaking in 1.0% NaOCl for 15 min and subsequently rinsing three times in sterile distilled water. Shoots were cultured on medium containing mineral salts, myo-inositol, vitamins and glycine betaine according to the MS formula (Murashige and Skoog, 1962) added with 3.0% sucrose and 8.5 g/L agar. The medium pH value was adjusted to 5.6–5.8 prior to autoclaving at 121 °C for 15 min. The initial uncontaminated shoots were then cultured on MS medium supplemented with 4.5 µmol/L BA for shoot multiplication for 8 wk. Subsequently, single shoots were transferred onto MS medium without any plant growth regulator for 6 wk before using as the explants source in all subsequent experiments. The incubation conditions were 16 h photoperiod (40 µmol/m²/s light intensity) at 25 ± 2 °C.

Shoot multiplication

Single, 1.0 cm-long shoot tips were cultured on the MS medium supplemented with a concentration of BA (0.0 µmol/L, 4.0 µmol/L, 8.0 µmol/L, 12.0 µmol/L, 16.0 µmol/L, 20.0 µmol/L and 40.0 µmol/L) or zeatin (0.0 µmol/L, 4.0 µmol/L, 8.0 µmol/L, 12.0 µmol/L and 16.0 µmol/L). The numbers of shoots and their lengths were measured after 4 wk.

Root induction

Single shoot tips 1.0 cm in length were cultured on the MS medium containing 0.0 µmol/L, 0.25 µmol/L, 5, and 7.5 µmol/L IBA or IAA for root induction. The numbers of roots and their lengths were recorded after 4 wk.

Callus induction

Single shoot tips 1.0 cm in length or the whole leaf of the first node wounded perpendicular to its midrib were cultured on the MS

medium supplemented with combinations of NAA (0.0 µmol/L, 20.0 µmol/L and 40.0 µmol/L) and BA (0.0 µmol/L, 4.0 µmol/L, 20.0 µmol/L and 40.0 µmol/L). The percentage of callus initiation was recorded. For shoot tip explants, the number of shoots, their length, the number of roots and their length were recorded after 4 wk. In addition, the callus was harvested and measured for length, width and height. For leaf explants, the dry weight of leaf producing callus and of leaf not producing callus in the control treatment were recorded after 2 wk. Since callus was unable to be separated from the leaf explant, it was reported in terms of the relative growth rate ($RGR = 100 \times \text{dry weight of treatment} / \text{dry weight of the control}$).

Statistical analysis

A completely randomized design (CRD) with 10 replicates was performed to determine the effect of BA and zeatin on shoot multiplication and the effect of IAA and IBA on root induction. A two-way factorial of two factors—NAA concentrations and BA concentrations—under a CRD was carried out for the callus induction. The experiment was performed with 10 and 6 replicates for shoot and leaf explants, respectively. Equal variances were tested using Levene's method. Where significant differences were found due to treatment, Tukey's B multiple range test was applied. Differences were considered significant at $p \leq 0.05$. All analyses were performed using the PASW Statistics 18 software (SPSS Inc.; Quarry Bay, Hong Kong).

Results

Shoot multiplication

Shoot tips of *L. camara* were cultured on MS medium supplemented with BA 0.0–40.0 µmol/L or 0.0–16.0 µmol/L zeatin for 4 wk. A high shoot production rate was found on MS medium added with 16.0 µmol/L, 20.0 µmol/L and 40.0 µmol/L BA, with the average shoot numbers being 4.9 shoots/explant, 7.1 shoots/explant and 6.8 shoots/explant, respectively. In addition, shoots cultured on the medium added with 8.0 µmol/L BA produced a number of multiple shoots not different from those produced using the media added with 16.0 µmol/L and 40.0 µmol/L BA. The media with 0.0 µmol/L and 4.0 µmol/L BA showed the lowest shoot number of one shoot/explant (Fig. 1a). Although these formulae produced the lowest production of shoots, they promoted shoot elongation to 3.4 cm and 3.8 cm long, respectively (Fig. 1b). Furthermore, the shoots found on the media added with 8.0 µmol/L, 16.0 µmol/L, 20.0 µmol/L and 40.0 µmol/L BA were healthy but short, with green expanded leaves (Fig. 2a).

The medium supplemented with zeatin at every concentration promoted shoot elongation but not shoot multiplication. There was no additional shoot formation on explants cultured on the media added with zeatin from 0.0 µmol/L to 12.0 µmol/L and only 1.3 shoots/explant were produced from shoot tips cultured on 16.0 µmol/L zeatin. The average shoot length of 4.9–6.7 cm derived from the treatments with 4.0–16.0 µmol/L zeatin was significantly longer than that obtained from the no-zeatin-treated control which produced the lowest shoot length (data not shown). However, shoots growing on both the control and zeatin-containing media were healthy with dark green expanded leaves (Fig. 2b).

Root induction

In vitro shoot tips of *L. camara* were cultured on MS medium added with IAA or IBA in the range 0.0–7.5 µmol/L to induce root formation at 4 wk. In the comparison between IAA and IBA at the

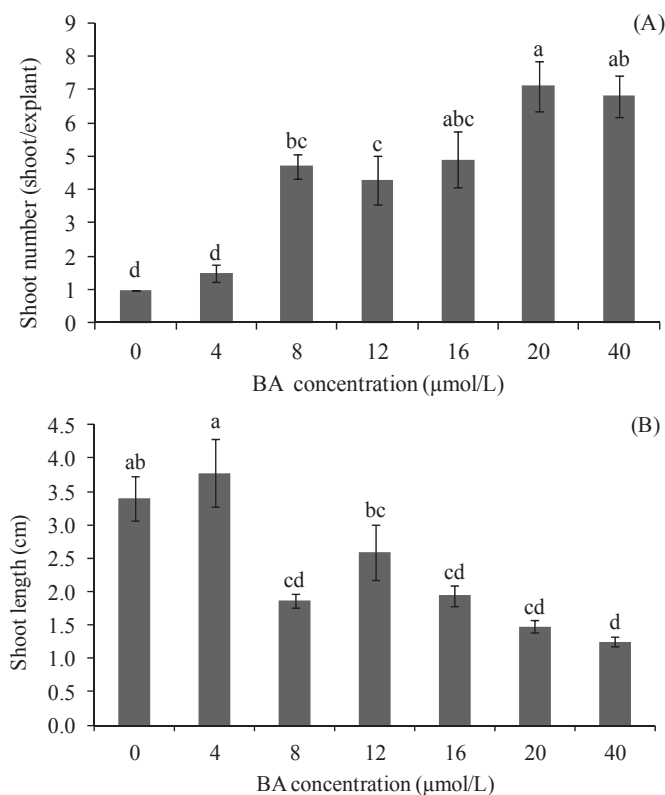


Fig. 1. (A) Shoot number and (B) shoot length of *Lantana camara* L. from shoot tip explants cultured on Murashige and Skoog medium added with N6-benzyladenine (BA) at 0.0–40.0 μmol/L after 4 wk. Error bars indicate SE; n = 10; different lower-case letters above bars indicate significant differences ($p \leq 0.05$).

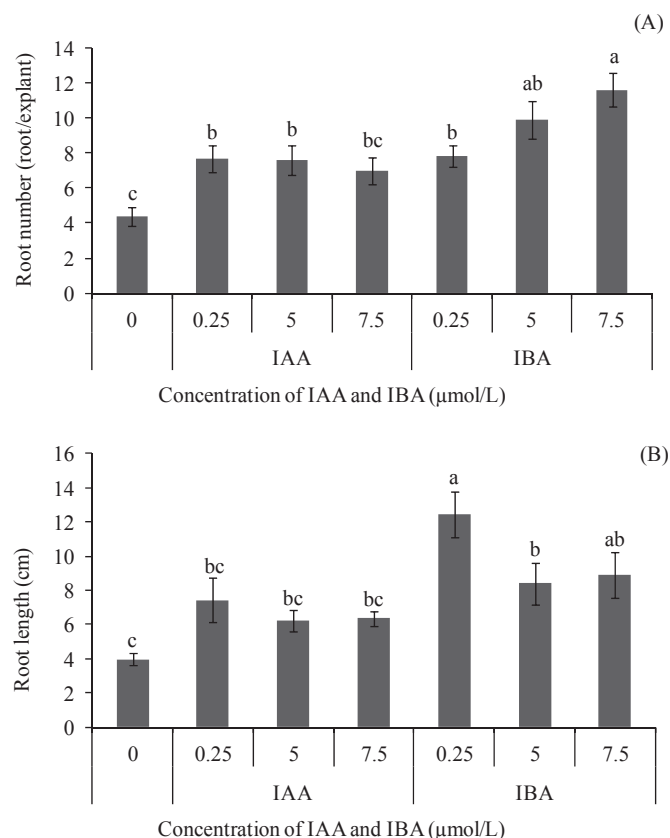


Fig. 3. (A) Root number and (B) root length of *Lantana camara* L. from shoot tip explants cultured on Murashige and Skoog medium added with indoleacetic acid (IAA) or indole-3-butyric acid (IBA) at 0.0–7.5 μmol/L after 4 wk. Error bars indicate SE; n = 10; different lower-case letters above bars indicate significant differences ($p \leq 0.05$).

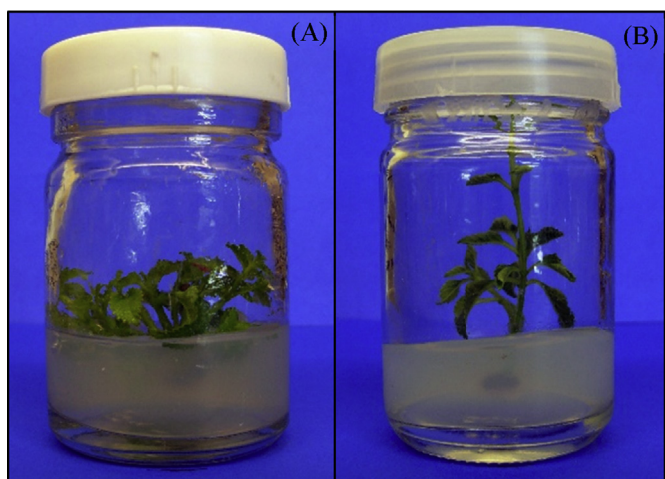


Fig. 2. Shoot multiplication of *Lantana camara* from shoot tip explants cultured on: (A) Murashige and Skoog (MS) medium supplemented with 16.0 μmol/L N6-benzyladenine; (B) MS medium supplemented with 16.0 μmol/L zeatin.

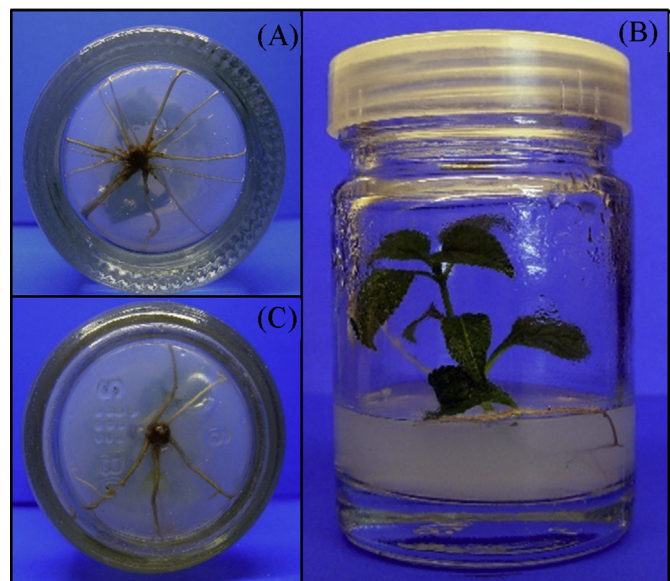


Fig. 4. Rooting of *Lantana camara* L. after 4 wk from shoot tip explants cultured on Murashige and Skoog medium added with (A, B) 7.5 μmol/L indole-3-butyric acid and (C) 7.5 μmol/L indoleacetic acid after 4 wk.

same concentration, high numbers of roots were found on the medium added with 5.0 μmol/L and 7.5 μmol/L IBA with 9.9 roots/shoot and 11.6 roots/shoot, respectively (Fig. 3a). The plantlets were healthy with dark green leaves and the roots were thick and long (Fig. 4a and b). The numbers of roots obtained from the control treatment using hormone-free MS was significantly lower than those obtained from 0.25 μmol/L to 5.0 μmol/L IAA and with every concentration of IBA tested. In addition, the numbers of roots found

on the media added with IAA at every concentration from 0.25 μmol/L to 7.5 μmol/L were not significantly different from those obtained from IBA 0.25 μmol/L and 5.0 μmol/L (Fig. 3a). The media added with 0.25 μmol/L and 7.5 μmol/L IBA promoted root

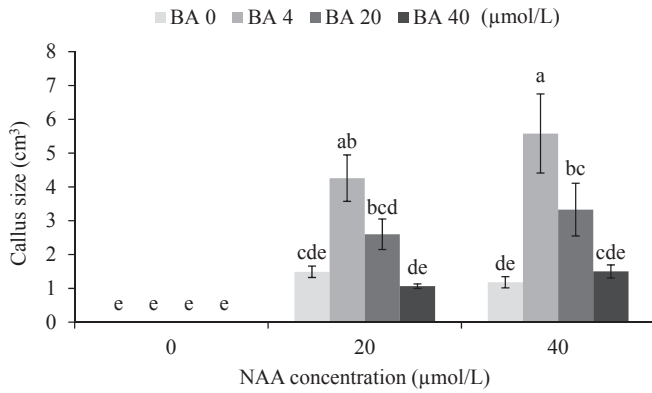


Fig. 5. Callus size of *Lantana camara* L. from shoot tip explants cultured on Murashige and Skoog medium supplemented with various concentrations of 1-naphthalene acetic acid (NAA) and N6-benzyladenine (BA) after 4 wk. Error bars indicate SE; n = 10; different lowercase letters above bars indicate significant differences ($p \leq 0.05$).

elongation to 12.5 cm and 8.9 cm long, respectively (Fig. 3b). All of the treatments added with IAA regenerated significantly shorter roots than those using 0.25 µmol/L IBA (Figs. 3b and 4c).

Callus induction

Shoot tips and leaf explants of *L. camara* were cultured on MS medium added with various concentrations of NAA in combination with BA. The results were recorded after 4 wk for the shoot tip explants and at 2 wk for the leaf explants.

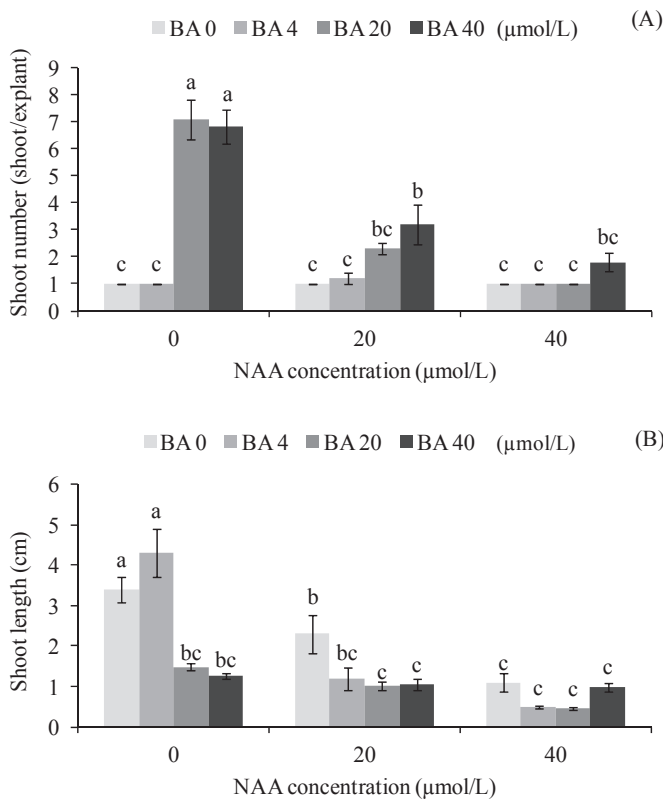


Fig. 6. (A) Shoot number and (B) shoot length of *Lantana camara* L. from shoot tip explants cultured on Murashige and Skoog medium supplemented with various concentrations of 1-naphthalene acetic acid (NAA) and N6-benzyladenine (BA) after 4 wk. Error bars indicate SE; n = 10; different lowercase letters above bars indicate significant differences ($p \leq 0.05$).

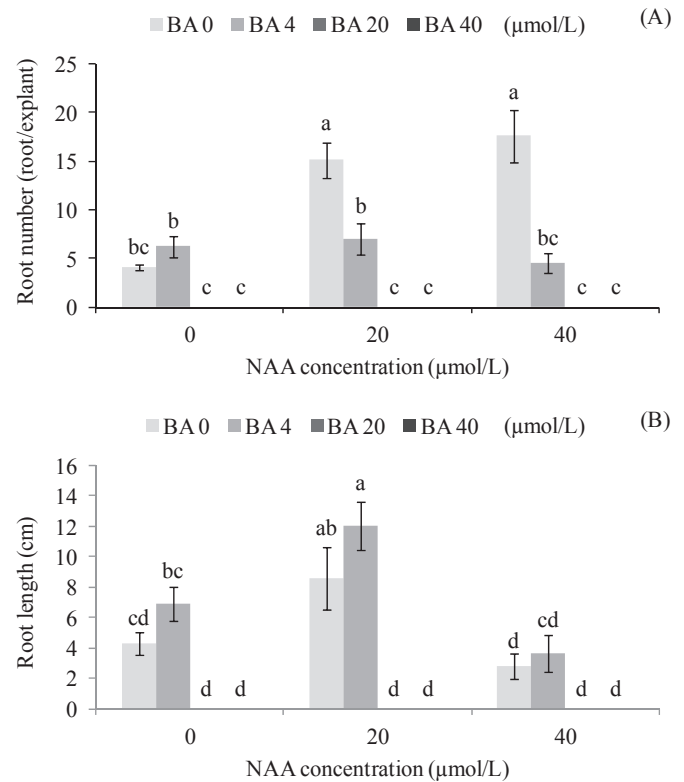


Fig. 7. (A) Root number and (B) root length of *Lantana camara* L. from shoot tip explants cultured on Murashige and Skoog medium supplemented with various concentrations of 1-naphthalene acetic acid (NAA) and N6-benzyladenine (BA) after 4 wk. Error bars indicate SE; n = 10; different lowercase letters above bars indicate significant differences ($p \leq 0.05$).

With the shoot tip explants, green compact callus formation was found on the media added with 20.0 µmol/L and 40.0 µmol/L NAA combined with BA at all concentrations (Figs. 5 and 9a). All the media formulae added with 0.0–40.0 µmol/L BA in combination with 20.0–40.0 µmol/L NAA showed 100% callus induction while the media added with only BA alone without NAA could not induce callus formation. Large-sized callus was found on the media added with 20.0 µmol/L and 40.0 µmol/L NAA in combination with 4.0 µmol/L BA with callus of 4.3 cm³ and 5.6 cm³, respectively. In addition, the callus formation found on the media added with

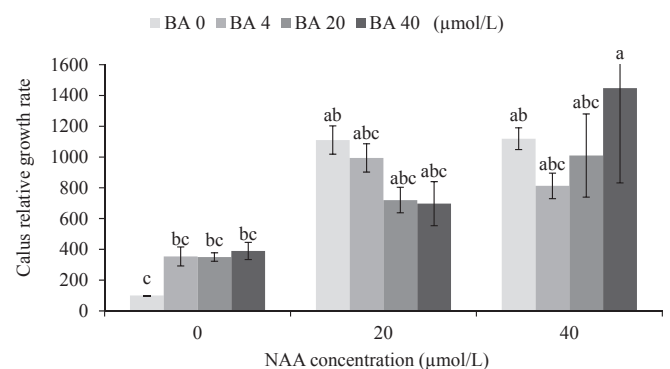


Fig. 8. Relative growth rate of *Lantana camara* L. callus derived from leaf explants cultured on Murashige and Skoog medium supplemented with various concentrations of 1-naphthalene acetic acid (NAA) and N6-benzyladenine (BA) after 2 wk. Error bars indicate SE; n = 6; different lowercase letters above bars indicate significant differences ($p \leq 0.05$).

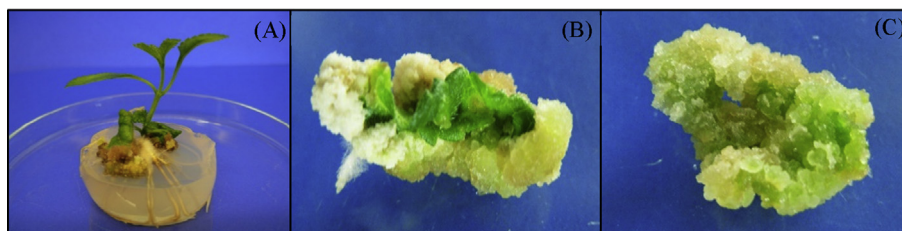


Fig. 9. *In vitro* growth and development of *Lantana camara* L.: (A) production of shoots, roots and callus at the basal end of a shoot (B) callus and roots formation on leaves, and (C) callus formation on leaves.

20.0 $\mu\text{mol/L}$ and 40.0 $\mu\text{mol/L}$ NAA combined with 20.0 $\mu\text{mol/L}$ BA was as large as that on the medium added with 20.0 $\mu\text{mol/L}$ NAA combined with 4.0 $\mu\text{mol/L}$ BA. Small-sized callus appeared on the media added with 20.0 $\mu\text{mol/L}$ and 40.0 $\mu\text{mol/L}$ NAA combined with 0.0 $\mu\text{mol/L}$ and 40.0 $\mu\text{mol/L}$ BA (Fig. 5). The callus grown on the top surface regularly turned brown and died (Fig. 9a).

In addition, shoots could develop from shoot tips on every treatment but root formation occurred only on the media added with 0.0 $\mu\text{mol/L}$ and 4.0 $\mu\text{mol/L}$ BA combined with every concentration of NAA. High numbers of shoot multiplication were found on the media added with 20.0 $\mu\text{mol/L}$ and 40.0 $\mu\text{mol/L}$ BA without NAA (Fig. 6a). Shoot growth was found to be enhanced by the media without NAA but added with 0.0 $\mu\text{mol/L}$ and 4.0 $\mu\text{mol/L}$ BA (Fig. 6b). On the other hand, the highest root numbers were found on the media added with 20.0 $\mu\text{mol/L}$ and 40.0 $\mu\text{mol/L}$ NAA only (Fig. 7a). Root length was found to be promoted by the media added with 20.0 $\mu\text{mol/L}$ NAA in combination with 0.0 $\mu\text{mol/L}$ and 4.0 $\mu\text{mol/L}$ BA (Fig. 7b).

From leaf explants, callus was able to be initiated on every treatment added with NAA and BA with the exception of the hormone-free MS medium. The dry weight of leaf-producing callus was recorded and presented in terms of the RGR because the callus was attached to the leaf explant. Based on the RGR values, supplementation of media with 20.0 $\mu\text{mol/L}$ and 40.0 $\mu\text{mol/L}$ NAA alone and 40 $\mu\text{mol/L}$ NAA in combination with 40.0 $\mu\text{mol/L}$ BA could significantly induce callus proliferation when compared to the control. Applying 40.0 $\mu\text{mol/L}$ NAA in combination with 40.0 $\mu\text{mol/L}$ BA could significantly enhance callus proliferation better than using BA alone. No significant difference among the treatments using NAA and BA in combination with the tested concentration range was found (Fig. 8).

Furthermore, roots regenerated on leaf explants were found on the media added with 20.0 $\mu\text{mol/L}$ and 40.0 $\mu\text{mol/L}$ NAA in combination with 0.0 $\mu\text{mol/L}$ or 4.0 $\mu\text{mol/L}$ BA (Fig. 9b). The translucent, yellow white and greenish compact callus was initiated and proliferated on every treatment with the exception of the medium containing no plant growth regulators (Fig. 9c).

Discussion

Many species in the family Verbenaceae have been reported to exhibit successful shoot multiplication when induced by various concentrations of BA, for example: 2.0 mg/L BA for *V. agnus-castus* (Balaraju et al., 2008), 1.0 mg/L BA for *V. negundo* L. (Stephen et al., 2010) and 3.0 mg/L BA for *P. serratifolia* L. (Ravinder Singh et al., 2011), as well as 0.1–0.7 mg/L BA for *L. camara* (Wao et al., 2013). These are related to the present study with *L. camara*, where BA was able to promote shoot proliferation but zeatin did not affect shoot induction. MS medium added with a high concentration of BA (16.0 $\mu\text{mol/L}$, 20.0 $\mu\text{mol/L}$ and 40.0 $\mu\text{mol/L}$) was found effective for shoot multiplication (4.9–6.8 shoots/explant). The report of Wao et al. (2013) indicated that a low BA

concentration produced 1–2 shoots. This suggested that the lower the concentration of BA used, the lower the number of shoots that were produced and a higher concentration of BA should reasonably induce higher shoot numbers. A BA concentration of 44.4 $\mu\text{mol/L}$ was considered to be too high since it caused shoot number reduction in *V. negundo* L. (Stephen et al., 2010). In the present study, no significant increase in shoot multiplication of *L. camara* was found when the concentration of BA was raised above 16.0 $\mu\text{mol/L}$. This result was consistent with the report on *V. negundo* L. that a BA concentration which was higher than the optimum level did not increase the shoot production rate (Usha et al., 2007; Stephen et al., 2010). In addition, shoot growth of *L. camara* was dependent on the BA concentration. It has been suggested that the application of cytokinin alone was enough for shoot multiplication; however, a higher cytokinin level may promote greater numbers of shoots but the growth of each shoot remained stunted (Bhojwani and Razdan, 1996). Shoots growing on the media added with 0.0–4.0 $\mu\text{mol/L}$ BA were long and healthy though no branching was evident, while the higher concentration of BA (8.0–16.0 $\mu\text{mol/L}$) resulted in shoot number induction with shoot length reduction but no significant difference. Their internodes and leaves were often found to be shorter and smaller when compared with those of shoots cultured on the lower concentration of BA. This was related to Stephen et al. (2010) who reported that increasing the concentration of BA up to 5.0 $\mu\text{mol/L}$ and 10.0 mg/L produced higher shoot numbers with shorter internodes and smaller leaves. This suggested that the best results for shoot proliferation with *L. camara* were using the medium added with 16.0 $\mu\text{mol/L}$ BA.

There have been many reports using a variety of auxins for root induction, including a combination of IBA and IAA for *V. negundo* L. (Sahoo and Chand, 1998; Usha et al., 2007), only IBA for *Vitex* spp. (Balaraju et al., 2008; Stephen et al., 2010) and only NAA for *P. serratifolia* L. (Ravinder Singh et al., 2011). In the present study, root induction of *L. camara*, similar to that of *V. negundo* reported by Balaraju et al. (2008) and Stephen et al. (2010) indicated that only a single auxin (IBA) was effective in producing healthy plantlets with numerous long roots without requiring a period greater than 4 wk (Fig. 4b). In contrast, *V. negundo* reported by Sahoo and Chand (1998) needed IBA in combination with IAA to induce root initiation, followed by subculturing to hormone-free, half-strength, MS medium for root elongation. On average, a high number of roots was produced from the shoots of *L. camara* on the medium added with 5.0 $\mu\text{mol/L}$ and 7.5 $\mu\text{mol/L}$ IBA which was similar to *V. negundo* L. where the optimum concentration of IBA was 4.9 $\mu\text{mol/L}$ (Usha et al., 2007). In addition, high root numbers of *L. camara* were found on the media added with 20.0 $\mu\text{mol/L}$ and 40.0 $\mu\text{mol/L}$ NAA; however, callus concomitantly occurred with roots at the basal end of shoots (Fig. 9a). This suggested that the best medium suitable for root induction in *L. camara* was the MS medium added with 7.5 $\mu\text{mol/L}$ IBA. Even though a high concentration of NAA could promote callus induction, it was not appropriate for root induction.

The type of explant always plays an important role in successful callus induction (Karunaratne et al., 2014). It has been suggested to select young explants close to the shoot apex, which is appropriate to induce dedifferentiation and form undifferentiated callus tissue (Victório et al., 2012; Karunaratne et al., 2014). This may be related to the regulation of phytohormones as endogenous auxins are mainly biosynthesized in the young developing leaves and the shoot apical meristem (Victório et al., 2012; Karunaratne et al., 2014); the state of dedifferentiation and redifferentiation depended on the balance between two plant hormones, auxin and cytokinin. The application of exogenous plant growth substances (auxin and cytokinin) of an intermediate ratio can promote callus induction. In addition, a high ratio of auxin:cytokinin and cytokinin:auxin induces root and shoot regeneration, respectively (Skoog and Miller, 1957; Ikeuchi et al., 2013). In the present research, the media containing BA (4.0–40.0 $\mu\text{mol/L}$) in combination with NAA (0.0–40.0 $\mu\text{mol/L}$) were able to induce callus formation on leaf explants (Fig. 9c), while the media added with only 20.0 $\mu\text{mol/L}$ and 40.0 $\mu\text{mol/L}$ NAA or 20.0 $\mu\text{mol/L}$ or 40.0 $\mu\text{mol/L}$ NAA combined with 4.0 $\mu\text{mol/L}$ BA produced callus with roots on leaf explants of *L. camara* (Fig. 9b). This indicated that the optimum ratio of auxin to cytokinin played a major role in the phenomenon of differentiation process. The combination of BA and NAA stimulated the formation of regenerative callus of *L. camara* similar to callus induction in teak (Widiyanto et al., 2005; Karunaratne et al., 2014). On the contrary, Saxena et al. (2013) reported that callus of *L. camara* L. could not be induced when explants exposed to the medium were added with some auxins or cytokinins individually as well as the combination of some auxins and cytokinins. The treatments comprised only 0.05 mg/L BA, only 0.01 mg/L or 0.05 mg/L IAA, 0.05 mg/L kinetin (KN) combined with 0.02 mg/L NAA, 0.02 mg/L KN combined with 0.02 mg/L NAA, only 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 0.01 mg/L BA combined with 0.05 mg/L IAA and 0.02 mg/L BA combined with 0.02 mg/L IAA. Callus was obtained only when stem and leaf explants were exposed to 0.2 mg/L 2,4-D, while the other media with BAP, IAA, KN combined with NAA, and BAP combined with IAA at various concentrations could not induce callus formation.

However, in the present results, the media containing 20.0 $\mu\text{mol/L}$ and 40.0 $\mu\text{mol/L}$ NAA combined with 4.0 $\mu\text{mol/L}$ BA always produced the largest callus size with roots at the basal end of the shoots. This was similar to the result with *Arabidopsis* in which shoots were incubated on callus inducing medium could form callus at pericycle cells adjacent to the xylem poles (Atta et al., 2009). Histological examination revealed that callus is not unorganized cells but has organized structures resembling the primordial of lateral roots. This suggested that the callus induction medium induces callus through the genetic pathway mediating lateral root initiation and this callus is not as dedifferentiated as previous thought (Atta et al., 2009; Ikeuchi et al., 2013).

In addition, the morphology differences in the callus may depend on the types of explants or plant growth regulators. In the present study, both shoot and leaf explants of *L. camara* cultured in all treatments produced yellow–white and greenish compact callus. On the other hand, callus of teak induced from leaf and stem explants on a similar medium of 0.02 mg/L 2,4-D presented different colours. The leaf explant produced brown–white, greenish, friable callus while the stem explant produced yellow–white, greenish friable callus (Saxena et al., 2013). Callus induction of Cavalcade (*Centrosema pascuorum* cv. Cavalcade) from shoot explants on various media showed differences in the callus morphology. White–greenish and compact callus of Cavalcade was able to form on media containing NAA in combination with BA, while friable, yellow callus formed on medium containing only NAA (Verapalakorn et al., 2012).

Notably, callus that had initiated at the basal end of shoots in all treatments where callus proliferated on the top surface, regularly turned brown and immediately died. On the other hand, callus initiated from leaf explants was able to grow continuously (Fig. 9c) though some treatments that produced large-sized callus developed a concomitant formation of roots on approximately 60–70% of shoot explants (Fig. 9b). This indicated that leaf explants were better than shoots for callus induction. In addition, media supplemented with only 4.0 $\mu\text{mol/L}$ BA or 20.0 $\mu\text{mol/L}$ or 40.0 $\mu\text{mol/L}$ BA combined with 0.0–40.0 $\mu\text{mol/L}$ NAA should be recommended for callus induction and proliferation.

Lantana camara has been reported as an effective, allelopathic chemical to inhibit the germination, growth and metabolism of crops, weeds, bryophytes and vegetables (Mishra, 2015). Its leaves and flowers exhibited strong biopesticide and bioherbicide properties to suppress a number of organisms including bacteria, fungi, aquatic and terrestrial weeds in its aqueous leachate/extract (Srivastava et al., 2011; Kalita et al., 2012; Reddy, 2013; Saxena et al., 2013). Finding the optimum plant growth substances for *in vitro* culture of *L. camara* in this research will be useful for further study in the production of allelochemicals from *in vitro* plant and callus. In addition, different plant growth regulators should be applied in order to examine callus induction. Various types of callus morphology may be produced and will be an effective option for the production of bioactive compounds and the evaluation of allelopathic properties.

This study suggested an effective protocol for the tissue culture technique of an important medicinal plant, *L. camara*. This should be fundamentally useful for further study in the production of bioactive compounds from *in vitro* plant material and micro-propagation for *ex vitro* conservation of *L. camara* and related species in the Verbenaceae.

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