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Adventitious Root Production of *Centella asiatica* in Response to Plant Growth Regulators and Sucrose Concentrations

Anna Pick Kiong Ling[1*] • Mee Feng Chin[1] • Sobri Hussein[2]

[1] Department of Bioscience, Faculty of Engineering and Science, Tunku Abdul Rahman University (UTAR), 53300 Setapak, Kuala Lumpur, Malaysia
[2] Agrotechnology and Bioscience Division, Malaysian Nuclear Agency, Bangi, 43000 Kajang, Selangor, Malaysia

Corresponding author: * **lingpk@utar.edu.my**

ABSTRACT

Centella asiatica is an important medicinal plant commonly used for wound healing purposes and as a brain tonic. A study was undertaken to investigate the effects of different plant growth regulators at various concentrations on adventitious root induction from the leaf and petiole explants. Full strength Murashige and Skoog (MS) medium supplemented with indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and α-naphthaleneacetic acid (NAA) at 0, 1, 3, 5, and 7 mg/L were used in this study. All treatments were kept in the dark and data collection was performed after 8 weeks of culture. No adventitious roots formed in the control medium devoid of any plant growth regulators (PGRs). Among the PGRs used, IBA showed the best adventitious root formation for both explants, followed by NAA, and IAA. The highest percentage of explants forming roots, number of roots per explant and longest roots formed from leaf explants was observed in 7 mg/L IBA. Meanwhile, IBA at 5 mg/L showed better rooting efficiency for petiole explants. Petiole explants were better than leaf explant for inducing adventitious roots. The effects of sucrose at 0, 1, 2, 3, 4, 5, 6, and 7% (w/v) on adventitious root induction from petiole explants were also assessed. No adventitious roots formed in MS media without sucrose. Sucrose at 4% and 5 mg/L of IBA showed the highest number of roots per explant and the longest roots.

Keywords: adventitious roots, asiaticoside, medicinal plant, secondary metabolites, wound healing
Abbreviations: IAA, indole-3-acetic acid; **IBA**, indole-3-butyric acid; **MS**, Murashige and Skoog medium; **NAA**, α-naphthaleneacetic acid; **PGR**, plant growth regulator

INTRODUCTION

Adventitious root formation is a key step in vegetative propagation of many crops and therefore of utmost economic importance (Scheres 2000). Adventitious roots of *Panax ginseng* induced by *in vitro* methods showed a high rate of proliferation and active secondary metabolism (Murthy *et al.* 2008). Unlike plant suspensions, which often produce very small amounts of secondary metabolites, root cultures can display high biosynthetic capabilities that are often comparable to those of normal roots (Kevers *et al.* 1999). Therefore, the cultures of adventitious roots are a potential source for the production of valuable plant secondary metabolites on a commercial scale (Min *et al.* 2007). Roots are the principle material for drug preparation in approximately 60% of the medicinal plants used in the traditional systems of medicine. Thus, the development of a root culture is highly advantageous, as it is also an alternative method for clonal propagation and germplasm conservation (Sudha and Seeni 2001).

Centella asiatica is one of the herbs that is claimed to possess various physiological effects. Traditional and herbal medicine practitioners have been using this plant in the treatment of different types of diseases for centuries after centuries (Hossain *et al.* 2005). The leaves or entire plant parts are boiled in water and this decoction is given in the treatment of leprosy where the glycoside asiaticoside is reported to be responsible for its use in treating leprosy (Chauhan 1999). The whole plant of *C. asiatica* has been proven to be beneficial in improving memory and it is also reported to improve general mental ability of mentally retarded children (Kumar and Gupta 2002). In 1990, the estimated annual requirement of *C. asiatica* was around 12,700 tonnes of dry biomass valued at Rs.1.5 billion (Tiwari *et al.* 2000). A large-scale and unrestricted exploitation of this natural resource coupled with limited cultivation and insufficient attempts for its replenishment, the wild stock of this species has been markedly depleted and now it is listed as threatened species by the International Union for Conservation of Nature and National Resources (IUCN) and an endangered species (Paramageetham *et al.* 2004).

There is an urgent need to conserve *C. asiatica* as it is now become an endangered species and the study on adventitious roots in this valuable germplasm is still very limited. In view of the importance of adventitious roots as well as the limited research that have been conducted on adventitious root induction of *C. asiatica*, thus, this study was conducted to determine the most suitable explant for adventitious roots induction. In addition, the effects of sucrose and plant growth regulators (PGRs) at various concentrations on adventitious root induction from different explants of *C. asiatica* were also investigated.

MATERIALS AND METHODS

Plant materials

Two-month old *C. asiatica* plants with kidney-shaped leaves were collected from Forest Research Institute Malaysia (FRIM) in Kepong, Selangor, Malaysia. In this study, four-week old leaves and petioles were used as explants.

Surface sterilization

Surface sterilization of the leaf and petiole explants were initiated by cleaning and washing thoroughly under running tap water for 30 min in order to wash off the external dust or contaminants. The leaves were then continuously shaken for 10 min and the petioles

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for 5 min in 20% (v/v) Clorox® containing 3 drops of Tween-20 (Amresco, USA) in a laminar flow bench. In order to remove the traces of Clorox®, the leaves and petioles were rinsed three times with sterile distilled water for 5, 10 and 15 min.

Culture media

Full strength MS medium (Murashige and Skoog 1962) was used in this study. The medium was prepared from each stock solution consisting of all the macronutrients, micronutrients, vitamins and Fe-NaEDTA. The auxins, indole-3-acetic acid (IAA) (Sigma-Aldrich, USA), indole-3-butyric acid (IBA) (ACROS Organics, Belgium) and α-naphthaleneacetic acid (NAA) (R&M Chemicals, Canada) at appropriate concentration, were added singly to the medium. Sucrose (Hamburg, Germany) was used as the carbon source in this study. The pH of the medium was adjusted to 5.8 ± 0.1 with 0.1 M NaOH or 0.1 M HCl using the pH meter prior to the addition of 0.8% (w/v) agar and autoclaved at 121°C for 15 min. Approximately 25 mL of the sterile medium were then poured into the 90 mm × 15 mm disposal sterile Petri dish (Greiner Bio-One, Australia).

Effects of different plant growth regulators at various concentrations

Two different types of explants namely, leaf and petiole explants were used to induce adventitious roots from *C. asiatica*. The leaf (5 mm^2) and petiole (0.5 cm) explants were aseptically excised and cultured on root induction medium. In this study, the effects of three different PGRs on adventitious root induction from leaf and petioles explants were studied. Full-strength MS medium supplemented with 5% (w/v) sucrose and various auxins (IAA, IBA and NAA) at 0, 1, 3, 5 and 7 mg/L were used as the roots induction medium for leaf and petiole explants. In this study, MS basal medium without any phytohormone served as the control. A total of 5 explants were placed on each culture medium. Each treatment, which consisted of 5 explants, was repeated three times. All root cultures were incubated at 25 ± 2°C in constant darkness.

Effects of carbon sources

As preliminary studies showed that petiole explants are the most suitable explant for adventitious root induction, petiole explants were selected for this part of the study. The effects of different amounts of sucrose on the induction of adventitious roots of *C. asiatica* were studied. The medium used was full-strength MS medium supplemented with 5 mg/L IBA and different concentrations of sucrose (0, 1, 2, 3, 4, 5, 6 and 7%, w/v). MS medium without any sucrose served as the control. A total of 5 explants were placed on each culture medium. Each treatment, which consisted of 5 explants, was repeated three times. All root cultures were incubated at 25 ± 2°C in constant darkness.

Data collection

The effects of various concentrations of different auxins on adventitious root formation for leaf and petiole explants and the effects of different concentrations of sucrose on adventitious root formation for petiole explants were recorded weekly The percentage of explants forming roots, the number of roots formed per explant, the average length of roots and the day of root formation were monitored for 8 weeks. The morphology and colour of the roots were also observed.

Statistical analysis

The experimental design was fully randomized. Data were analyzed statistically by Analysis of Variance (ANOVA) followed by the Tukey's HSD test, with the level of significance 5% ($p < 0.05$) to compare the differences among the different treatment means, if any. Statistical analysis was performed using the SPSS for Windows (Version 15.0).

RESULTS AND DISCUSSION

Effects of different auxins at various concentrations on leaf explants

Leaf explants showed a lower rooting efficiency than petiole explants with different PGRs at various concentrations. Adventitious roots could only be induced in some PGR treatments. Only IBA at 1 and 7 mg/L, and NAA at 1, 3 and 5 mg/L were able to induce adventitious roots successfully. For IBA treatments, adventitious roots were formed through both the direct and indirect pathways. At 1 mg/L, adventitious roots were formed directly from the explants whilst in 7 mg/L IBA callus was induced on the leaf explants after two weeks of culture prior to the formation of adventitious roots. Adventitious roots formation through the indirect pathway has been reported in *Panax ginseng* whereby the adventitious roots were induced through the callus phase in the presence of 3.0 mg/L IBA (Ali *et al*. 2005). The inhibition of direct organogenesis could be explained by the fact that a high concentration of auxins inhibited root growth and development and resulted in root apical dormancy (Chao *et al*. 2006). In this study, by comparing adventitious roots induction within the IBA treatments, regardless of the pathway, it was found that MS medium with 7 mg/L IBA showed the highest percentage of explants forming roots, number of roots per explant and length of roots (**Fig. 1**). Approximately 8.3 ± 8.3% of the leaf explants cultured formed roots with 4.8 ± 7.1 roots forming per explant and 1.0 ± 1.2 cm roots observed in the treatment containing 7 mg/L IBA (**Table 1**). Although the treatment with 1 mg/L IBA showed a lower rooting efficiency than 7 mg/L IBA, 1 mg/L IBA only took 7.3 ± 12.7 days to form adventitious roots whereas adventitious root initiation in 7 mg/L IBA took 22.8 ± 22.8 days.

Apart from IBA, NAA treatments were also able to induce adventitious roots from leaf explants but at a lower rooting efficiency compared to IBA. NAA at 1 mg/L showed

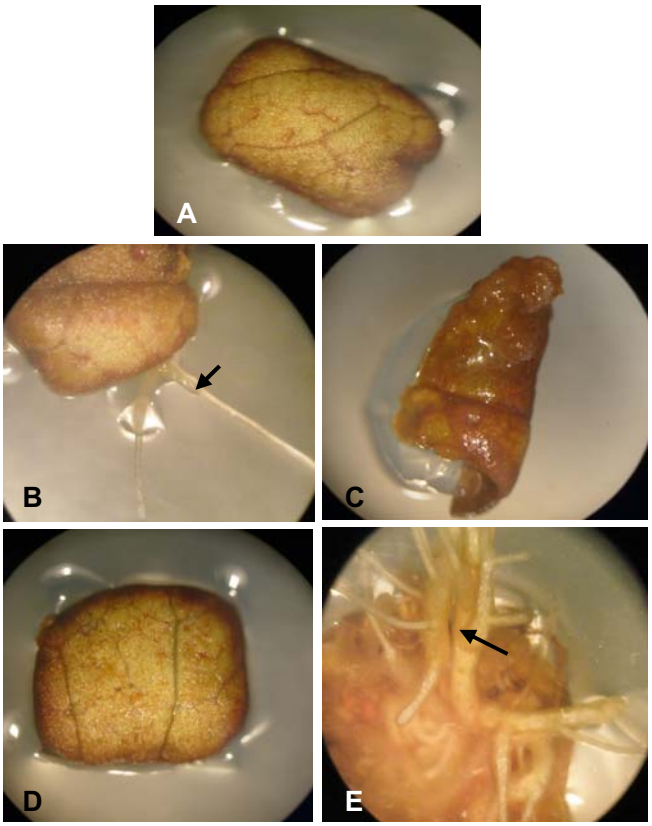

Fig. 1 Effects of IBA at various concentrations on adventitious root induction from leaf explants of *C. asiatica* after 8 weeks of culture. (**A**) 0 mg/L, (**B**) 1 mg/L, (**C**) 3 mg/L, (**D**) 5 mg/L, (**E**) 7 mg/L. Black arrow shows the formation of adventitious roots.

Table 1 Effects of different auxins at various concentrations on the adventitious roots induction from leaf explants of *C. asiatica* after 8 weeks of culture.

Plant growth regulators	Concentration (mg/L)	Day of root formation (Mean ± SD)	Explants forming roots (%)	№ of roots per explant	Length of adventitious roots (cm)
IAA	0	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	1	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	3	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	5	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	7	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
IBA	0	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	1	7.3 ± 12.7 a	2.2 ± 2.2 a	1.0 ± 1.0 a	0.6 ± 0.6 a
	3	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	5	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	7	22.8 ± 22.8 a	8.3 ± 4.8 a	4.8 ± 4.1 b	1.0 ± 0.7 b
NAA	0	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	1	10.2 ± 17.6 a	5.6 ± 5.6 a	1.0 ± 1.0 a	0.4 ± 0.4 a
	3	16.3 ± 28.3 a	2.2 ± 2.2 a	0.7 ± 0.7 a	0.3 ± 0.3 a
	5	18.7 ± 32.3 a	2.2 ± 2.2 a	0.3 ± 0.3 a	0.2 ±0.2 a
	7	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a

Values represent means ± standard deviations for three replications.
Values followed by the same letter are not significantly different at the $p < 0.05$ level, according to the Tukey's HSD test.
(-) = no growth,

the highest percentage of explants forming roots with 5.6 ± 9.6% (**Table 1**). Treatments containing 3 and 5 mg/L NAA showed the same percentage of explants forming roots (2.2 ± 3.9%) whereas no adventitious roots formed in the NAA treatment at the highest concentration, i.e. 7 mg/L. Treatment with 1 mg/L NAA also recorded the highest value in terms of the number of roots per explant and the length of roots, which were 1.0 ± 1.7 and 0.4 ± 0.7 cm, respectively (**Table 1**). When the concentration of NAA was increased from 3 to 5 mg/L, the number of roots per explant and the length of roots formed decreased. No adventitious roots formed in the treatment with 7 mg/L NAA. The initiation of adventitious roots was fastest in medium containing 1 mg/L NAA taking only 10.2 ± 17.6 days. The explants took a longer time to initiate the formation of adventitious roots as the concentration of NAA was increased from 3 to 5 mg/L.

With a low percentage of adventitious root formation, this study revealed that the leaf explants of *C. asiatica* were not a suitable explant in the induction of adventitious roots. This statement was supported by a study done by Gao *et al.* (2005) whereby leaf explants of *Panax notoginseng* cultured on solid MS basal medium supplemented with different concentrations of PGRs failed to initiate root organogenesis. In contrast, other studies proved that leaf explants were effective in root induction. The leaf explants of *Decalepis arayalpathra* (Sudha and Seeni 2001) and *Cichorium litybus* L. cv. 'Focus' (Nandagopal and Kumari 2007) incubated in total darkness in half-strength MS medium supplemented with an optimum combination of IBA (0.5 mg/L) and NAA (0.2 mg/L) showed rapid induction of roots and higher growth after a period of 6 weeks. Similarly, young leaf explants of *Quercus robur* 'Fastigiata' cultured in darkness on medium with 4.0 mg/L NAA and 0.4 mg/L BA were strongly reactive and first formed callus followed by adventitious roots (Pierik *et al.* 1997).

Effects of different auxins at various concentrations on petiole explants

Petiole explant was observed to be a better explant in the induction of adventitious roots from *C. asiatica* as compared to the leaf explant. This result was in accordance with the study by Burritt and Leung (1996) whereby the petiole sections excised from *Begunia x erythrophylla* plants grown *in vitro*, were highly organogenic, with shoots and roots arising directly from epidermal cells. The regenerative capacity of rose petioles could be ascribed to basipetal transport of endogenous auxins and/or carbohydrates, and position of the regenerative target cells (Pati *et al.* 2006). Root organogenesis from petioles cultured on different auxins at all concentrations was preceded with an intermediate callus phase. The plant development through organogenesis is the formation of organs either *de novo* or adventitious in origin. Whole plant regeneration via organogenesis is a monopolar structure and it develops procambial strands which establish a connection with the pre-existing vascular tissue dispersed within the callus or cultured explants (Chawla 2002).

IBA was the best PGR in the induction of adventitious roots from the petiole explants of *C. asiatica* compared to NAA and IAA. All the IBA treatments successfully induced adventitious roots. As the concentration of IBA was increased from 1 to 5 mg/L, the percentage of explants forming roots, the number of roots per explant and the length of roots formed also increased (**Fig. 2**). Treatment with 5 mg/L of IBA recorded 43.3 ± 30.9% of explants forming roots, 11.1 ± 11.2 roots per explant and 1.2 ± 0.8 cm, which marked the highest value among all the concentrations of IBA tested (**Table 2**). When the concentrations of IBA were

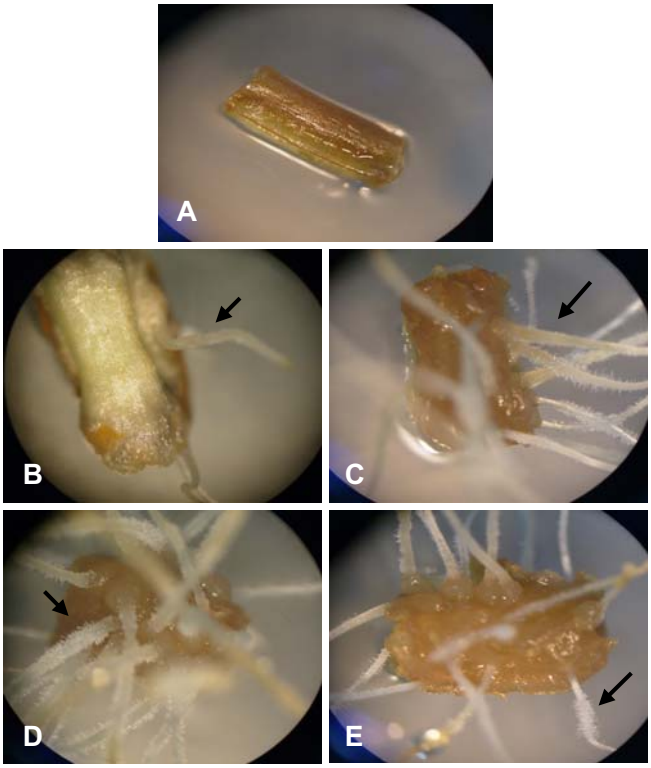

Fig. 2 Effects of IBA at various concentrations on adventitious root induction from petiole explants of *C. asiatica* after 8 weeks of culture. (**A**) 0 mg/L, (**B**) 1 mg/L, (**C**) 3 mg/L, (**D**) 5 mg/L, (**E**) 7 mg/L. Black arrow shows the formation of adventitious roots.

Table 2 Effects of different auxins at various concentrations on the adventitious roots induction from petiole explants of *C. asiatica* after 8 weeks of culture.

Plant growth regulators	Concentration (mg/L)	Day of root formation (Mean ± SD)	Explants forming roots (%)	№ of roots per explant	Length of adventitious roots (cm)
IAA	0	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	1	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	3	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	5	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	7	10.7 ± 18.5 a	2.2 ± 2.2 a	0.3 ± 0.3 a	0.2 ± 0.2 a
IBA	0	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	1	21.3 ± 21.0 a	20.4 ± 13.0 a	2.2 ± 1.7 ab	0.6 ± 0.5 a
	3	35.7 ± 10.9 a	34.8 ± 14.1 a	6.4 ± 2.2 ab	1.1 ± 0.1 a
	5	34.1 ± 4.7 a	43.3 ± 17.8 a	11.1 ± 6.5 b	1.1 ± 0.4 a
	7	23.6 ± 21.4 a	15.6 ± 8.7 a	3.4 ± 3.0 ab	0.6 ± 0.3 a
NAA	0	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	1	32.0 ± 28.8 a	25.9 ± 16.1 a	1.7 ± 0.8 ab	0.4 ± 0.2 a
	3	27.3 ± 25.3 a	19.8 ± 15.8 a	2.3 ± 1.2 ab	0.5 ± 0.2 a
	5	24.2 ± 21.0 a	19.4 ± 15.5 a	1.7 ± 1.2 ab	0.4 ± 0.2 a
	7	45.2 ± 3.8 a	28.9 ± 9.3 a	3.4 ± 1.3 ab	0.5 ± 0.2 a

Values represent means ± standard deviations for three replications.
Values followed by the same letter are not significantly different at the $p < 0.05$ level, according to the Tukey's HSD test.
(-) = no growth

increased from 5 to 7 mg/L, the percentage of explants forming roots, the number of roots per explant and the length of roots formed were decreased. IBA at the concentration of 1 mg/L took only 21.3 ± 21.0 days to form adventitious roots which were the earliest root initiation in the treatment with various concentrations of IBA. In the study done by Nath and Buragohain (2003), profuse rooting of *C. asiatica* was obtained in full-strength MS medium supplemented with 2 mg/L IBA. In addition to that, Banerjee *et al.* (1999) and Tiwari *et al.* (2000) also found a promotory effect of IBA in rooting of *C. asiatica*. According to Siddique *et al.* (2006), IBA was found to be more effective in the induction of roots without inducing callus in *Hemidesmus indicus* and *Vitex negundo*. IBA at 5 mg/L was optimal in the adventitious roots formation from petiole explants in this study. Pierik *et al.* (1997) similarly found a low rooting response of *Quercus robur* '*Fastigiata*' in the range of 0 to 0.3 mg/L IBA, but a gradual increase occurred from 0.3 to 5.0 mg/L and 5 mg/L was optimal.

Currently, IBA is the most widely used auxin to stimulate the rooting process in cuttings because of its high ability to promote root initiation as well as its weak toxicity and great stability in comparison to NAA and IAA (Qaddoury and Amssa 2004). Several hypotheses have been postulated to explain the rooting efficacy of IBA. IBA is more stable than IAA under various light and temperature conditions, both in solution and *in vivo* (Nissen and Sutter 1990; Nordstrom *et al.* 1991). Differences in transport, uptake, or metabolism might also contribute to the superior activity (Epstein and Ludwig-Muller 1993). Alternatively, a specific IBA to IAA ratio may be important for development, and the application of exogenous IBA might shift the balance to promote root development (Zolman *et al.* 2000). In addition to that, IBA has a slower rate of conjugation than IAA, so that the free IBA required to induce rooting will be available over a longer period of time (Krisantini *et al.* 2006).

The second best PGR in adventitious roots induction was NAA. The highest percentage of explants forming roots, the number of roots per explant and the length of roots formed were observed in the MS medium containing NAA at the concentration of 7 mg/L, which were 28.9 ± 16.0%, 3.4 ± 2.2 roots, and 0.5 ± 0.4 cm, respectively (**Table 2**). The lowest percentage of explants forming roots (19.4 ± 26.8%) and the lowest number of roots per explant (1.7 ± 2.1) was recorded in the treatment with 5 mg/L NAA whilst 1 mg/L NAA formed the shortest roots from the petiole explants, which was only 0.4 ± 0.3 cm. The treatment with 5 mg/L NAA recorded the fastest roots initiation where it took 24.2 ± 21.0 days to form adventitious roots. Although 7 mg/L NAA resulted in the highest percentage of explants forming roots, the number of roots per explant and the length of roots formed, this treatment was the slowest (45.2 ± 3.8 days) in the initiation of adventitious roots. NAA is known to affect and stimulate rooting more than IAA (Arteca 1996). This statement was supported by the study done by George *et al.* (2008a) whereby NAA was the most effective auxin in root induction of *Baliospermum montanum* and this can be correlated to the faster uptake of NAA as compared to IAA. There have been numerous reports that NAA is involved in the initiation of adventitious roots and that division of roots initially is dependent either upon the exogenous or endogenous auxin (Ercisli *et al.* 2002; Haynes and Samagula 2003).

IAA was found to be the least effective PGR in the rooting response of *C. asiatica*. In the IAA treatments, adventitious roots were only successfully formed in the MS medium containing 7 mg/L IAA whereas other concentrations of IAA were not able to induce the adventitious roots. IAA at the concentration of 7 mg/L showed 2.2 ± 3.9% of explants forming roots, 0.3 ± 0.6 roots induced per explant and 0.2 ± 0.4 cm roots (**Table 2**). Root initiation in 7 mg/L IAA took 10.7 ± 18.5 days for the initiation of adventitious roots. However, there were studies that showed IAA was a better PGR in the formation of adventitious roots. In the study conducted by de Klerk *et al.* (1997), IAA was the preferable auxin for *in vitro* rooting of apple 'Jork 9' shoots compared to IBA and NAA during adventitious root formation *in vitro* in *Malus* 'Jork 9'. The treatment of IAA in *Rosa hybrida* L. was found to increase the root number significantly at higher concentrations of IAA (Azadi 2007).

Effects of different sucrose concentrations on petiole explants

The availability of carbohydrates is often considered exclusively as an energetic requirement and carbon skeleton source to drive root development (Correa *et al.* 2005). According to the previous study done by Hossain *et al.* (2005) on the effect of different carbon sources on *in vitro* regeneration of *C. asiatica*, the best root induction was observed on medium containing 0.2 mg/L IBA and 3% sucrose as carbon source as compared to sugar, gur, glucose and maltose while half-strength MS media supplemented with 0.5 mg/L IBA and 2% sucrose was reported optimum by Patra *et al.* (1998). Sucrose is usually hydrolysed partially or completely in the medium into the component monosaccharides glucose and fructose, which are taken up by the plant tissues partly through active transport, and partly through passive permeation (Taylor and van Staden 2001). Vinterhalter *et al.* (2001) previously reported the superiority of sucrose as compared to glucose and fructose in the *in vitro* culture of carob tree, *Ceratonia siliqua*. The general

Table 3 Effects of different sucrose concentrations on the adventitious roots induction from petiole explants of *C. asiatica* after 8 weeks of culture.

Sucrose concentration (% w/v)	Day of root formation (Day ± SD)	Explants forming roots (%)	№ of roots per explant	Length of adventitious roots (cm)
0	-	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
1	27.3 ± 23.7 a	13.9 ± 10.0 a	1.7 ± 0.9 a	0.5 ± 0.3 ab
2	12.6 ± 21.7 a	6.7 ± 6.7 a	0.7 ± 0.7 a	0.2 ± 0.2 ab
3	29.4 ± 12.6 a	43.3 ± 15.8 a	7.0 ± 3.0 a	0.8 ± 0.2 abc
4	31.7 ± 12.0 a	17.0 ± 3.5 a	19.5 ± 3.3 b	1.6 ± 0.4 c
5	28.5 ± 12.9 a	33.3 ± 12.5 a	8.5 ± 1.2 a	0.9 ± 0.1 abc
6	40.8 ± 8.6 a	38.9 ± 14.7 a	6.7 ± 3.9 a	0.7 ± 0.1 abc
7	43.5 ± 3.5 a	30.4 ± 4.1 a	4.6 ± 1.1 a	1.1 ± 0.2 bc

Values represent means ± standard deviations for three replications.
Values followed by the same letter are not significantly different at the $p < 0.05$ level, according to the Tukey test.
(-) = no growth

superiority of sucrose over glucose for the culture of organised plant tissues such as isolated roots may be on account of the more effective translocation of sucrose to apical meristems (George *et al.* 2008b).

The MS medium without addition of sucrose (0% w/v) showed no adventitious roots formation until 8 weeks of culture. Perez *et al.* (2004) stated that most in vitro cultures are not able to proliferate properly without an exogenous supply of carbohydrates. The highest percentage of explants forming roots (43.3 ± 27.3%) was observed in the treatment containing 3% of sucrose (**Table 3**). The MS medium containing 4% of sucrose showed the highest number of roots per explant and the longest roots formation, which were 19.5 ± 5.7 roots and 1.6 ± 0.8 cm, respectively. Meanwhile, the treatment containing 2% sucrose recorded the lowest value in terms of percentage of explants forming roots, the number of roots per explant and the length of roots. Although the medium supplemented with 2% sucrose was less efficient in the rooting efficiency, this treatment only took 12.6 ± 21.7 days to initiate the adventitious roots. Sucrose at 7% was the slowest (43.5 ± 3.5 days) in the initiation of roots formation.

The optimal sucrose concentration for the induction of adventitious roots was varied with different species of plants. In the study done by Calamar and de Klerk (2002) on the effect of sucrose on adventitious root regeneration in apple, the sucrose concentration influenced the number of adventitious roots, whereby increasing the sucrose concentration up to 7% resulted in increased rooting. The results also showed that during adventitious root formation, applied sucrose was used as a source of energy and building blocks. Meanwhile, the highest amount of phenols and flavonoids were accumulated in the adventitious roots of *Echinacea angustifolia* cultured in 5% sucrose (Wu *et al.* 2006). Yu (2000) reported that for adventitious root cultures of *Panax ginseng*, a relatively higher sucrose concentration (5%) is more favourable for biomass development, whereas Zhong *et al*. (1995) found that 4.5% (w/v) sucrose was the best for the production of anthocyanin in *Perilla frutescens*.

CONCLUSIONS

Plant roots function much more than in just nutrient and water uptake only. Roots have also been recognized as the major contributors to the production of secondary metabolites. The advantages of root cultures are that they show a greater genetic stability and retain differentiation while exhibiting growth rates comparable to those of plant cell suspensions. In addition to that, unlike plant suspensions which often produce very small amounts of secondary metabolites, root cultures also can display stable metabolic productivity and high biosynthetic capabilities that are often comparable to those of normal roots. Thus, more research can be done for the optimization of the chemical and physical parameters for the cultivation of roots to produce higher yields of secondary metabolites.

Apart from that, further studies on the induction of adventitious roots can be carried out using liquid medium. Liquid medium stimulate better cell growth of plant tissue as compared to solid medium. The ability to grow root cultures from many plant species in isolation and to manipulate root metabolism, allows the isolation and characterization of enzymes involved in root-specific pathways, cloning of the corresponding genes and understanding of pathway regulation which provides the necessary background to predictably manipulate root biosynthetic potential in the whole plant, as well as in scaled-up root cultures (Flores *et al*. 1999). The ability of adventitious or hairy roots to grow to high density and to produce significant amount of secondary metabolites makes them a suitable system for large-scale culture in bioreactor (Min *et al*. 2007). The improvement of adventitious root culture system through the use of bioreactor seems to be reliable way for the production of pharmaceutically and nutraceutically important metabolites.

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