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Full Length Research Paper

Somatic embryogenesis and root regeneration in *Hyoscyamus niger* L. for the production of hyoscyamine

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Embryogenic calli could be induced from the leaf, petiole and root pieces of *Hyoscyamus niger* using different induction medium. The embryogenic calli produced were different in term of colour and morphology. Small globular somatic embryos could only be produced from the leaf explants cultured on Murashige and Skoog (MS) medium supplemented with 6 mg/L picloram. The globular embryos increased in size and developed into torpedo, heart shape or bipolar embryos accompanied by root formation after cultured onto MS basal medium for one week then followed by two weeks of culture in the maturation medium (MS + 1.0 mg/L BA). More roots could be regenerated from the leaf-derived somatic embryos when they were transferred into liquid MS medium without any plant growth regulator. Our result indicated that the leaf-derived embryogenic callus induced on solid MS supplemented with 6.0 mg/L of picloram did not show the presence of hyoscyamine. However, the *H. niger* roots regenerated from the somatic embryos showed the presence of hyoscyamine. Although the somatic embryos failed to germinate to complete plantlets, the regenerated roots from the somatic embryos could be mass propagated to obtain hyoscyamine, the main tropane alkaloid produced in *H. niger*.

Key words: Embryogenic callus, hyoscyamine, *Hyoscyamus niger,* root regeneration, somatic embryos.

INTRODUCTION

Hyoscyamus niger L. (Solonaceae) is an important medicinal plant widely distributed in China, Afghanistan, India, Japan, Korea, South West Asia, North Africa and throughout Europe (Sajeli et al., 2006). It is commonly known as black henbane or stinking nightshade and is commercially cultivated for its alkaloid compounds. All parts of the plant are poisonous but it is used for various treatments such as ear and eye inflammation, rheumatism, treatment of ulcers, cough due to tuberculosis, motion sickness, asthma and fever (Ramoutsaki et al., 2002). It is also used for religious and ceremonial purposes and as narcotics and analgesic. In Turkey, the seeds of *H.* niger is used as folk medicine. The seeds were boiled in water and added in steam bath as a treatment for conjunctivitis (Tuzlaci and Aymaz, 2001).

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The plant produces two important alkaloids, hyoscyamine and scopolamine. Both are used for dilating pupils for fundoscopy and are used in anesthesia to prevent bradycardia and to decrease bronchial and salivary secretions (Jung et al., 2001). Husain (1983) reported that the leaves contained 0.01-0.1% of the total alkaloids with approximately 75% hyoscyamine and 25% scopolamine (hyoscine).

Lai (2003) had proven that tropane alkaloids could be obtained via micropropagated plantlets and root cultures of *H. niger*. Plantlets had been successfully regenerated in *H. niger* from anther and pollen culture (Raghavan, 1975; Nagmani and Raghavan, 1983). Indirect somatic embryogenesis from callus had also been reported but with very low frequency of plant regeneration (Cheng and Raghavan, 1985). The induction of direct embryogenesis in *H. niger* was reported by Tu et al. (2005) using mature zygotic embryos of seeds. However, somatic embryogenesis from vegetative tissue and root regeneration from the somatic embryos in *H. niger* were not reported until today.

Theoretically, a culture initiated from a single explant can produce an unlimited number of embryos. Thus *in vitro* somatic embryogenesis generally use as an alternative for large scale production of plants or plant organs for the production of secondary metabolites. However, somatic embryogenesis is influenced by explant source and genotype. A wide range of plant tissues has been used as explant source from which to obtain somatic embryos. The choice of explant is considered to be an important factor in the induction of somatic embryos. In the present study, we describe the induction of somatic embryos from different plant parts of *H. niger* and to evaluate whether somatic embryos can be used for the regeneration of roots for the production of hyoscyamine.

MATERIALS AND METHODS

Plant materials

Seeds of *H. niger* were purchased from Richters (Canada). The seeds were washed with tap water for 30 min and were disinfected by two stage surface-sterilization technique with Clorox®, a commercial bleach solution containing 5.3% sodium hypochlorite. In the first stage, the seeds were surface-sterilized for 15 min in 10% (v/v) Clorox® containing 2 or 3 drops of Tween 20 followed by rinsing with sterile distilled water three times. The seeds were again immersed in 5% Clorox® for 5 min followed by rinsing again three times with sterile distilled water in the second stage. The seeds were inoculated on MS basal medium (Murashige and Skoog, 1962) for germination. The seed cultures were incubated at 25 ± 2°C and 24 h photoperiod with light intensity of 32 µE m^{-2} s^{-1} photosynthetic photon flux density. The 4 weeks old *in vitro* seedlings were used as plant materials for subsequent studies.

Induction of embryogenic callus

Leaf, root and petiole explants of 4 weeks old *in vitro* seedlings were used for embryogenic calli induction. Leaf (0.5 x 0.5 cm), root and petiole explants (1 cm) were transferred to MS basal medium supplemented with 0.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/L 4-amino-3,4,6-trichloro picolinic acid (Picloram) to determine the optimum concentration and type of auxin for the induction of embryogenic callus. Fresh biomass of the embryogenic callus was determined after 30 days of inoculation in order to determine the best embryogenic callus induction medium for each type of explants. The callus formed was observed closely at two days intervals for any change in the morphology. Eight experimental units were used for each combination medium in a 6 x 6 factorial block design. The data was analyzed using Factorial 2-Way ANOVA followed by mean comparison using Tukey HSD at p = 0.05.

Development of somatic embryos

An embryogenic callus mass of 100 mg induced from the leaf explants on MS medium supplemented with 6.0 mg/L picloram were transferred to the MS medium without any plant growth regulator for a week. After which they were transferred onto the maturation medium, MS + 1.0 mg/L 6-benzylaminopurine (BA), the optimum em-bryos maturation medium determined in our previous study (data not shown). The number of globular and torpedo shape embryos were determined after 30 days of culture.

Effect of solid and liquid medium on development of somatic embryo

Embryogenic mass with globular embryos (100 mg) were transferred to MS medium without plant growth regulators (PGRs) for a week and then subcultured into gelled medium (solidified with 7.5 g/l agar, Algas, Chile) or liquid MS medium supplemented with 1.0 mg/L BA, the embryos maturation medium. The liquid cultures were placed on orbital shaker and were continuously agitated at 120 revolution per minute (rpm). Ten experimental units were used for each solid and liquid medium. The embryogenic callus biomass, number of globular and torpedo shape embryos were recorded after 30 days of culture. The data was analyzed using student t-test at p = 0.05.

Histological study of embryogenic callus and different stages of somatic embryos development

For histological studies, embryogenic callus with different stages of development were fixed in formalin acetic acid (FAA) for 48 h before dehydrated in ethyl alcohol and tertiary butyl alcohol (TBA) series, embedded in paraffin wax (MP 58°C), sectioned at 10 µm and fixed on glass slides before stained with Safranin and Fast Green. Sections were mounted in Distyrene Plasticizer Xylene (DPX) mixture mountant and the prepared slides were viewed under a light microscope (Olympus SZ-PT Stereo Light Microscope).

Root regeneration

Somatic embryos (100 mg) were transferred into 50 ml Erlenmeyer flasks containing 25 ml MS basic medium. Eight experimental units were used for the study. The cultures were placed in the culture room maintained at 25 ± 2ºC with continuous lighting with intensity of 32 µE m^{-2} s^{-1} photosynthetic photon flux density. The formation of roots was evaluated after 4 weeks of culture.

Detection of hyoscyamine

Methods of extraction of hyoscyamine and sample preparation were modified from Kartal et al. (2002). The embryogenic callus and root samples were taken out from the cultures and were air dried until constant weight at 25ºC. The dried samples were macerated into powder using a mortar and pestle. The macerated powder (1.0 g) was then placed into the extraction thimble and extracted with 125 ml methanol in a Soxhlet apparatus for two hours. The extract was evaporated at 50ºC to dryness using a rotary evaporator (Eyela Rotary Vacuum Evaporator N-N Series and Eyela Digital Waterbath SB-651, Tokyo Rikikai Co. Ltd.) coupled with a water pump (Eyela Aspirator A-3S, Tokyo Rikikai Co. Ltd.). The pH of the residue was adjusted to 3 with 2 M HCl and then filtered. The filtrate was extracted 3 times with 2 ml of petroleum ether. The alkaloids were extracted 4 times with 5 ml of chloroform from the alkali aqueous solution (pH 10) using ammonia. The chloroform was evaporated to dryness and the residue was dissolved in 1 ml of dichloromethane. The extract was ready for injecting into the gas chromatograph column. L-Hyosyamine (Sigma Chemical Co.) was used as standard. The standard was accurately weighed (1.0 mg) into a 10 ml volumetric flask and dissolved and filled up to volume with dichloromethane. Standard solution of l-hyoscyamine and (1.0 g/ml) samples were prepared in the dichloromethane. The standard solution and samples prepared were analyzed by Gas Chromatography Mass Spectrometry (GCMS). One (1.0) µl of the standard and samples were injected into the GCMS column. GCMS was carried out on a Varian-Chrompack 3800 gas chromatograph

Table 1. Effects of MS medium supplemented with 2,4-D and Picloram on embryogenic callus induction (g ± s.d) from the leaf, petiole and root explants of *Hyoscyamus niger* after four weeks of culture.

2,4-D (mg/L)	Picloram (mg/L)					
	0	2.0	4.0	6.0	8.0	10.0
Leaf explants						
0	0.00g	0.44bcdef	0.29cdefg	1.10±0.53a	0.70±0.14b	0.55±0.25bcd
2.0	0.16 ± 0.11 efg	0.25 ± 0.18cdefg	0.22 ± 0.12cdefg	0.56±0.27bcd	0.27±0.20 cdefg	0.57±0.22bc
4.0	0.10 ± 0.04 fg	0.21 ± 0.11cdefg	0.16 ± 0.12efg	0.51±0.25bcde	0.14±0.12fg	0.14±0.11fg
6.0	0.12 ± 0.05 fg	0.19 ± 0.13efg	0.10 ± 0.04fg	0.16±0.01efg	0.12±0.10fg	0.03±0.02g
8.0	0.10 ± 0.14 fg	0.21 ± 0.31cdefg	0.24 ± 0.17cdefg	0.22±0.16cdefg	0.15±0.08fg	0.15±0.11fg
10.0	0.05 ± 0.02g	0.11 ± 0.06fg	0.13 ± 0.09fg	0.30±0.26cdefg	0.11±0.04fg	0.21±0.20defg
Petiole explants						
0	0.00±0.00h	0.41± 0.21ab	0.70±0.26a	0.39±0.29ab	0.31±0.10abc	0.26±0.19abcd
2.0	0.05 ±0.01efgh	0.23±0.05abcde	0.20± 0.08bcdefg	0.26±0.09abcd	0.15±0.11cdefgh	0.11±0.04defgh
4.0	0.04±0.03efgh	0.01±0.05defgh	0.22± 0.15abcdef	0.32±0.09abc	0.05±0.02efgh	0.04±0.02efgh
6.0	0.04±0.02efgh	0.05±0.03efgh	0.15± 0.11cdefgh	0.09±0.08defgh	0.00±0.00h	0.01±0.06defgh
8.0	0.02±0.09gh	0.02±0.01fgh	0.07± 0.04defgh	0.03±0.03gh	0.00±0.00h	0.02±0.02gh
10.0	0.00± 0.00h	0.06±0.06efgh	0.16± 0.06cdefgh	0.15±0.17cdefgh	0.04 ±0.01gh	0.14±0.08gh
Root explants						
0	0.00±0.00b	0.15±0.11b	0.06±0.04b	0.05±0.04b	0.17±0.17b	0.14±0.08b
2.0	0.01±0.02b	0.02±0.02b	0.02±0.01b	0.04±0.03b	0.06±0.04b	0.57±0.22a
4.0	0.10± 0.04b	0.21±0.11b	0.16±0.12b	0.51±0.25ab	0.14±0.12b	0.14±0.11b
6.0	0.12±0.05b	0.19±0.13b	0.10±0.04b	0.16±0.01b	0.12±0.10b	0.03±0.02b
8.0	0.10±0.14b	0.21±0.31ab	0.24±0.17b	0.22±0.16b	0.15±0.08b	0.15±0.11b
10.0	0.05±0.02b	0.11±0.06b	0.13±0.09b	0.30±0.26ab	0.11±0.04b	0.21±0.20 ab

Means values of embryogenic callus mass derived from each explant type followed by different alphabet were significantly different (Tukey HSD, p = 0.05).

coupled to a Saturn 2000 mass detector. A mass spectrometer with an ion trap detector in full scan (80-325 amu) under electron impact ionization (70 eV) was used. The chromatographic column for the analysis was a Chrompack WCOT-Fused Silica CP-Sil 5 CB capillary column (30 m x 0.25 µm). The carrier gas used was helium at a flow rate of 1 ml/min. Standard solution and samples of 1 µl were injected and analyzed with the column held initially at 125ºC with a 10ºC/min heating ramp and subsequently kept at 250ºC for 5 min. The injection was performed in splitless mode at 270ºC. The chromatograms and mass spectra of the standard were equivalent with that of the Mass Spectrometer Wiley library for authenticity. These were then used as the basis for comparison of hyoscyamine in the plant samples. The chromatograms and mass spectra of the samples were then matched with that of the standards for compare-son to detect the presence of hyoscyamine at a similar retention time.

RESULTS AND DISCUSSION

Induction of embryogenic callus

The capacity for embryogenic callus induction was strongly affected by type of explants used. Different explants of *H. niger* were also found to require different amount and different type of PGRs for callus induction. The leaf explants cultured on MS medium supplemented with 6.0 mg/L picloram produced the highest amount of embryogenic callus (1.10 ± 0.53 g) after four weeks of culture. For the petiole explants, embryogenic callus was best induced on MS medium supplemented with 4.0 mg/L picloram. For both the leaf and petiole explants, the amount of embryogenic callus produced on their respec-tive best callus induction medium was significantly different from the amount of calluses induced in other MS medium supplemented with different concentrations of 2,4-D and picloram. The addition of 2,4-D into MS medium containing picloram was in fact not necessary for the induction of embryogenic callus from these two explants. As the concentration of 2,4-D added into the medium increased, the amount of calluses induced were reduced. However, the root explants was found to require the addition of 2,4-D in the culture medium for the induc-tion of embryogenic calli. MS medium supplemented with 2.0 mg/L 2,4-D and 10.0 mg/L picloram was found to be the best callus induction medium for the root explants (Table 1). The induction of embryogenic calli in *H. niger* was hence influenced by the concentration and type of auxin supplemented into the culture medium. Our result indicated that MS medium supplemented with picloram induced more embryogenic callus in *H. niger.*

Figure 1. Globular somatic embryos formed on the outer layer of callus from leaf explant after 4 weeks of culture on MS supplemented with 6.0 mg/L picloram (A); Whitish green friable embryogenic callus from petiole explant (B); hard and compact whitish brown embryogenic callus from root explant (C).

The embryogenic callus derived from different explants was different in term of colour and morphology (Figure 1). The leaf-derived embryogenic callus was white with mixture of compact and friable type of callus. Small globular somatic embryos appeared from the outer layer of the callus after two week of culture. Whitish green and friable embryogenic callus formed at the cutting edge of the petiole explants after two weeks of culture. The embryogenic callus induced from the root explants was hard, compact and whitish (light) brown in colour. The leaf and petiole explants started to become swollen and remained white or green while the root explants started to turn brown after one week of culture. Embryogenic callus started to appear at the cutting edges that came in contact with the callus induction medium at the second week of culture for the leaf and petiole explants while the growth of little callus that formed in root explants was slow. This study thus indicated that formation of embryogenic callus was different using different explants of the same plant species.

In the present study, out of the three types of explants used for induction of embryogenic callus (leaf, petiole and root), the leaf explants was considered as the best explant for the production of embryogenic callus based on embryogenic cell biomass and the formation of globular embryos. Therefore, embryogenic calli derived from leaf explants cultured on MS supplemented with 6.0 mg/L picloram were used for further experiments to trace the development of somatic embryos.

Development of somatic embryos

After transferring onto the MS basal medium for a week followed by the maturation medium (MS + 1.0 mg/L BA), the globular embryos that were existed on the leaf-derived callus increased in size and developed into torpedo, heart shape or bipolar embryos and root after two weeks of culture. An average of 21.8 ± 7.6 globular shaped embryos, 7.1 ± 1.7 torpedo shaped embryos and 4.8 ± 1.7 roots were produced from the 100 mg embryogenic callus mass after two weeks of culture. Primary roots emerged from the embryos after four weeks of transferring into the maturation medium. According to Kintzios et al. (2004), maturation of embryos involved passage through globular, oblong, heart and torpedo stages and the torpedo embryos had the potential to regenerate to plantlets. All the formation stages of somatic embryos of globular, heart-shaped and torpedo embryos were observed in *H. niger* when cultured on MS supplemented with 1.0 mg/L BA after pre cultured on MS basal medium for one week. Incubating the embryogenic callus of *H. niger* on MS medium without auxin for a week might be the triggering effect for further development of somatic embryos. However, in our study only roots were produced from the somatic embryos without the formation of shoots even though after they were transferred onto the maturation medium containing the cytokinin. Singla et al. (2007) reported that the expression of embryogenic-related genes might be staggered depending on the duration of the auxin present in the medium and most

Table 2. Effect of solid and liquid MS medium supplemented with 1.0 mg/L BA on development of somatic embryos of *H. niger.*

MS + 1.0 mg/L BA	Mean Weight (g) of embryogenic mass (± s.d)	Mean No. of globular embryo (± s.d)	Mean No. of torpedo embryo (± s.d)	Mean No. of roots (± s.d)
Solid	2.07 ± 0.51 a	22.6 ± 12.8 a	6.0 ± 1.9 b	5.6 ± 3.8 a
Liquid	0.50 ± 0.10 b	4.4 ± 2.5 b	2.0 ± 0.8 a	10.3 ± 4.7 b

Means within the same column followed by the same alphabet are not significantly different using student t-test at p = 0.05.

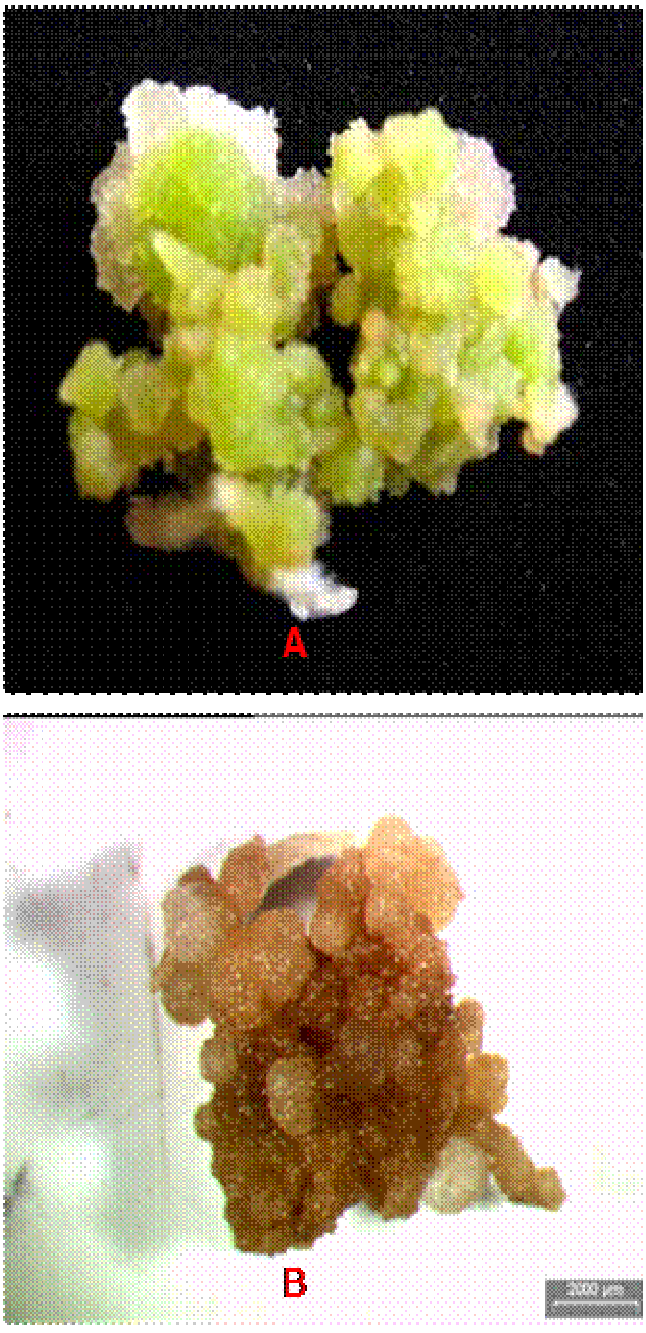

Figure 2. Somatic embryos of *H. niger* after four weeks of culture on MS + 1.0 mg/L BA (A) green somatic embryos on gelled medium; (B) brown somatic embryos in liquid medium.

genes involved in somatic embryogenesis were activated during auxin treatment thus showed the importance of auxin for embryogenic callus induction in this study. The presence of auxin during the callus induction stage might have also induced rhyzogenesis and hence promoted root formation instead of shoot production in *H. niger* embryos.

Effect of solid and liquid medium on development of somatic embryos

Results indicated that solid MS medium supplemented with 1.0 mg/L BA promoted better growth of embryogenic callus (2.07 ± 0.51 g), more globular (22.6 ± 12.8) and torpedo shaped embryos (6.0 ± 1.9) were formed compared to liquid MS medium with the same constituent. Moreover, the gelled medium could support more than 200 times embryogenic growth from the initial mass of 100 mg while the liquid medium only stimulated five times the growth. However, liquid medium was found to be effective in root induction. The liquid medium could induce the somatic embryos to regenerate into more roots (10.3 ± 4.7) than the solid media (5.6 ± 3.8) (Table 2).

All globular and torpedo shaped embryos remained green in color and increased in size with time on solid MS medium. Further development of somatic embryos from globular embryos stage to torpedo shape embryo could also be found in the liquid medium as early 8 days of culture but after that the somatic embryos became brown in colour and their growth was slow compared to the solid medium (Figure 2).

Histological study of leaf-derived embryogenesis callus of *H. niger*

Histological sections of the embryogenic callus mass of *H. niger* showed cells of different sizes and types. There were cells with relatively dense cytoplasm, generally small invisible vacuoles with nucleus and cells with unclear cytoplasm and large vacuoles.

The globular embryos were composed of cells with dense cytoplasm, thick cell walls and large nuclei. These cells showed mitotic and meristematic activity and formed a meristematic cell zone (Figure 3 A). Both periclinal and

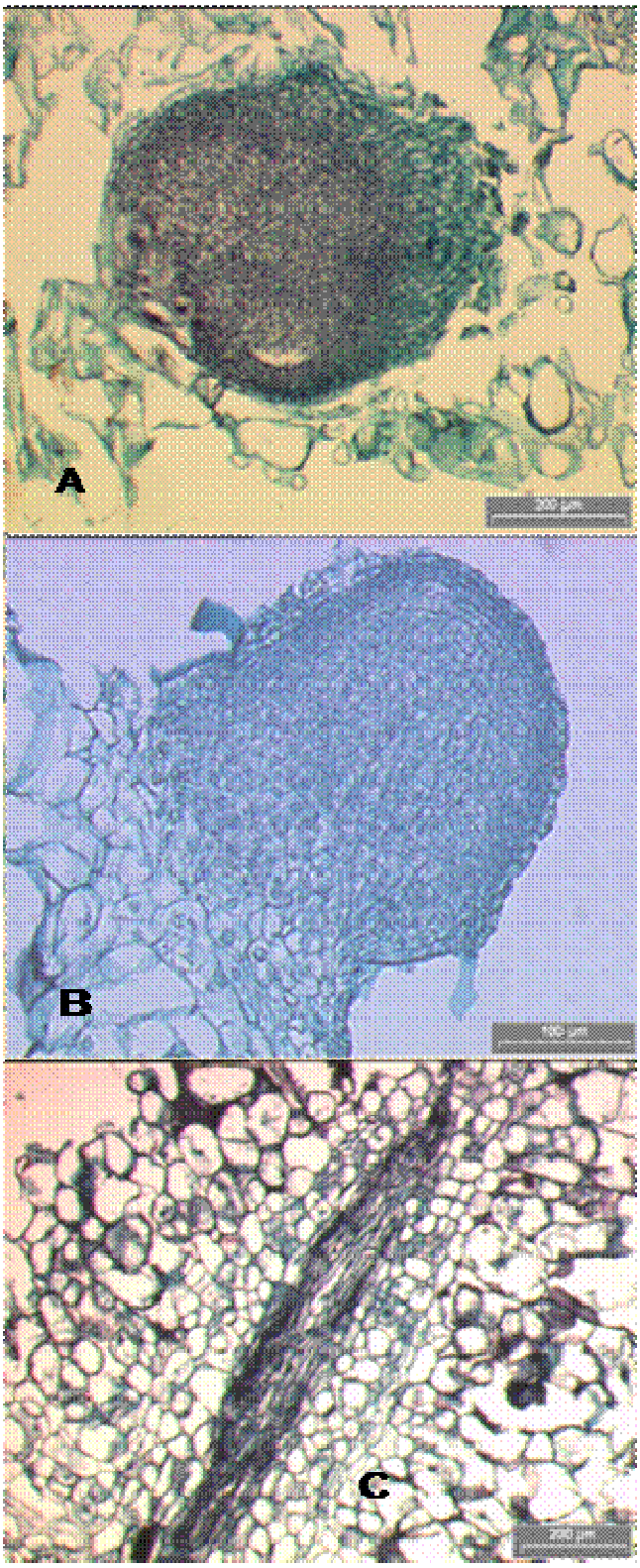

Figure 3. Histological study of *H. niger* embryogenic callus mass. (A) Somatic embryos with dense cytoplasm and meristematic zone; (B) Globular somatic embryo that emerged from proliferating meristematic cells within the embryogenic mass; (C) Differentiation resulted in the formation of root-like structures with vascular bundles.

anticlinal cells division could be observed in the meristematic zones and these cells were characterized with dense cytoplasm compared to the surrounding cells which could be considered as the first stage of somatic embryo differentiation. Some of the meristematic cells showed signs of proliferation at the epidermal layer. The epidermal cells of the embryogenic masses elongated vertically and at certain points, developing somatic embryo emerged (Figure 3 B). The embryogenic cells of *H. niger* were found to have small granules that were believed to be starch granules. Loiseau et al. (1998) suggested the starch granules were used for somatic embryo initiation and development. According to Plata et al. (1991), starch was metabolized in the embryogenic tissue to supply energy for localized mitotic and metabolic activities. Starch accumulation was also observed by Ribas et al. (2000) in the embryogenic cells of *Aspidosperma polyneuron*. Somatic embryos could differentiate to form root-like structures as early as two weeks after culture. More meristematic cells elongated and developed into primary root structures. Histology study showed that the root had vascular bundle that newly developed. These vascular bundles could also be found in embryogenic callus that were pre cultured on MS medium for one week and been transferred to MS + 1.0 mg/L BA for four weeks (Figure 3C).

Groups of cells with unclear cytoplasm, large vacuoles and non-nucleated were determined as non embryogenic cells. A similar phenomenon was observed by Thiruvengadam et al. (2006) and these cells could suppress the proliferation of the embryogenic cells. Therefore the embryogenic tissue should be separated from the non embryogenic tissue for continuation of embryo proliferation and further development process. In the present study, induction of embryogenic callus confirmed by histological study which indicated the indirect embryogenesis process occurred in *H. niger* using the leaf explants.

Root regeneration

Embryo cells that were transferred to the MS liquid medium promoted root regeneration. Formation of primary roots from the globular somatic embryos appeared during the first week of culture. The whitish brown somatic embryos started to form green root after second week. From third week onwards, only root elongation was observed without further development of embryos (Figure 4). The root grows up to 3.0 cm after 4 weeks of culture. Prolonged culture of the somatic embryos in the liquid medium only induced elongation of roots. Only roots were produced from the somatic embryos of *H. niger* without plantlets formation in the basic MS medium. According to Che et al. (2006) failure of somatic embryos to regenerate to form plantlets could be due to down-regulation of genes controlling plant regeneration. This could also be occurring in *H. niger* somatic embryos.

Figure 4. Root formation from the somatic embryos of *H. niger* after the embryogenic cells were transferred to MS liquid medium after three weeks of culture.

Detection of hyoscyamine in somatic embryos and root cultures of *H. niger*

The total ion chromatogram (TIC) scan of the Hyoscyamine standard produced a good clear peak at t_R (retention time) 34.31 min. The ion fragments obtained from the Hyoscyamine standard were *m/z* (rel. int.): 300 [M]+ (1), 141 (5), 126.2 (11), 125.2 (100), 122.1 (4), 97.3 (14), 95.3 (28), 92.3 (20), 83.4 (37), 78.4 (19), 69.4 (10), 68.5 (31) (Figure 5A). When compared with the mass spectrum in the Wiley database library of the mass spectrometer for authenticity, the mass spectrum of the prepared standard gave a 97% match as well as a confirmatory compound structure match. This was then used as the reference spectrum for hyoscyamine in the extracts of the embryogenic callus and root samples of *H. niger*. Our result indicated that the leaf-derived embryogenic callus induced on solid MS supplemented with 6.0 mg/L of picloram did not show the presence of hyoscyamine. However, TIC of the extracts of the *H. niger* roots regenerated from the somatic embryos showed the presence of hyoscyamine at the same retention time as the standards at t_R 34.31 min. Hyoscyamine detected in the root samples produced ion fragments of *m/z* (rel. int.): 300 [M]+ (1), 141 (6), 126.2 (22), 125.2 (100), 122.1 (4), 97.3 (17), 95.3 (41), 92.3 (37), 83.4 (25), 78.4 (29), 69.4 (13), 68.5 (38) (Figure 5B). When compared with the mass spectrum in the Wiley database library of the mass spectrometer for authenticity, the mass spectrum of the prepared standard gave a 90% match as well as a confirmatory compound structure match.

Conclusion

Somatic embryos of *H. niger* could be produced from the leaf-derived embryogenic calli. Although the somatic embryos failed to germinate to form plantlets, the regene-

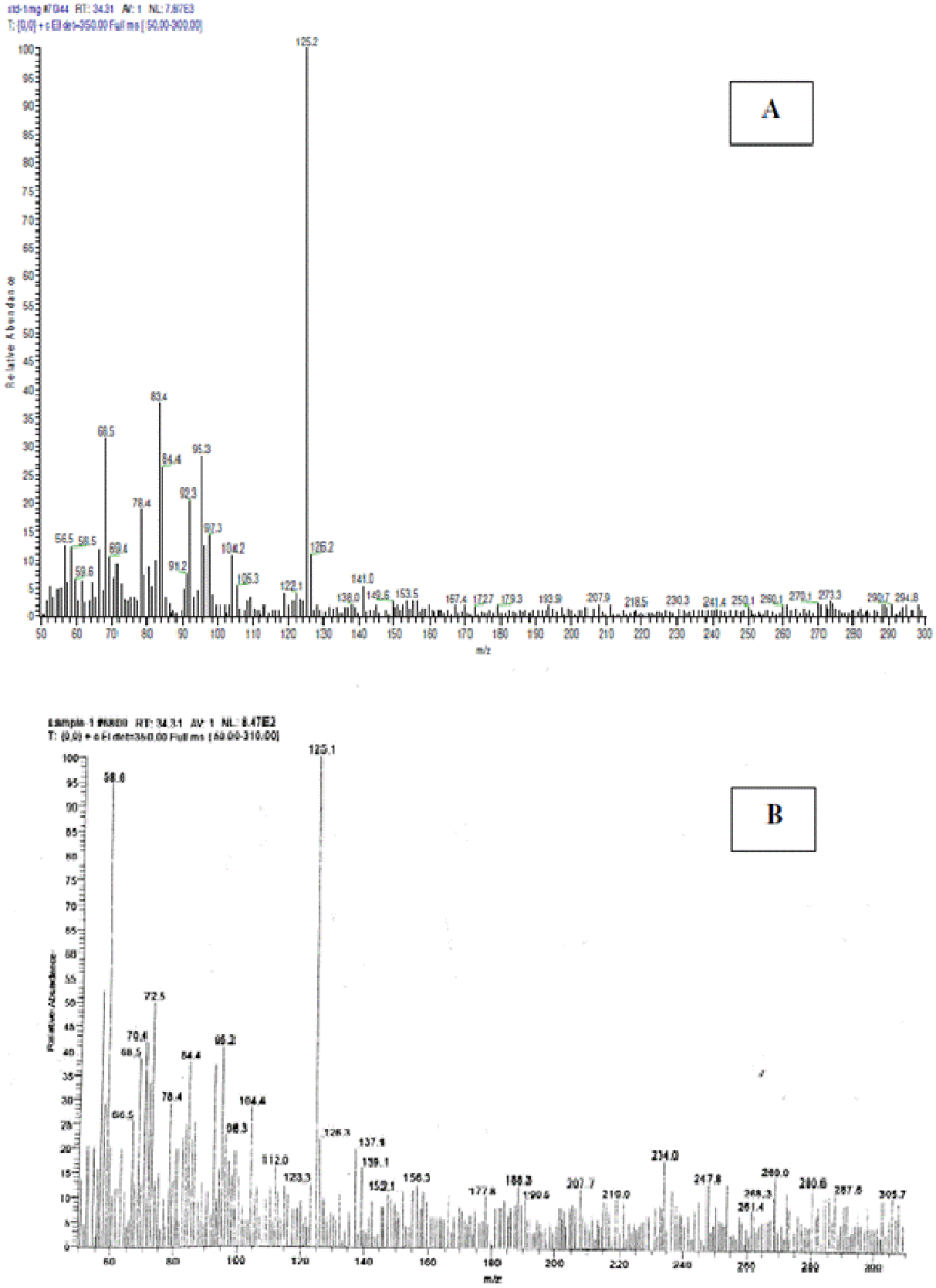

Figure 5. The mass spectrum of Hyoscyamine standard (A) and the mass spectrum of Hyoscyamine in the extract of roots regenerated from the somatic embryos of *H. niger*

rated roots from the somatic embryos could be mass propagate to obtain hyoscyamine, the main tropane alkaloid produced in *H. niger*.

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