Rhizogenesis in Cell Suspension Culture ~~from~~ of Mango Ginger *(Curcuma amada Roxb.)*: A Source of Isosorbide and n-Hexadecanoic Acid

A~~bstract~~Summary

Mango ginger (*Curcuma amada* Roxb.~~)~~ belongs to the monocotyledonous family Zingiberaceae. It ~~is~~ provides ~~commonly known as mango ginger and used as a spice and~~a valuable medicine, as well as a spice. In this study, adventitious roots of *C. amada* ~~have been~~were successfully established from cell suspension culture. ~~The highest percentage of a~~Adventitious root production was highest from ~~obtained from~~ friable callus-~~-~~derived cell suspension culture. ~~The~~ optimal culture conditions ~~of~~ for adventitious root production were ~~optimized~~ determined;~~and the~~ maximum adventitious root production was obtained in half-~~-~~strength MS liquid medium containing 0.3 mg ~~L-1~~ indole-3-butyric acid ~~L-1~~ ~~along with~~and 3% ~~of~~ sucrose after 5 weeks of culture. ~~Among the different initial inoculum density, t~~The ~~best~~ optimal initial inoculum density ~~culture condition~~ for root growth ~~occurred at~~was 10 g fresh weight~~FW of initial inoculum density~~. Gas chromatography-mass spectrometry ~~GC-MS~~ analysis revealed that ~~the *in vitro* raised~~ adventitious roots generated *in vitro* ~~containing~~ contained two valuable bioactive compounds, isosorbide and n-hexadecanoic acid. The~~se results~~ ~~outcome of the present work~~ will be helpful ~~for~~ in advancing the large-~~-~~scale cultivation of adventitious roots for the production of valuable bioactive compounds.

Introduction

Curcuma amada Roxb. (mango ginger) of the Zingiberaceae family is a unique perennial rhizomatous herb, which morphologically resembles ginger and has a flavour of raw mango (*Mangifera indica*). There are 68 volatile aromas, and more than 130 chemical constituents in the mango ginger rhizome. The plant's aromatic smell is mainly attributed to the presence of car-3-ene and cis-ocimene compounds, which are used in food, beverages, cosmetics, and medicines (Dutt and Tayal, 1941; Gholap and Bandyopadhyay, 1984; Rao *et al.*, 1989; Choudhury *et al.*, 1996; Srivastava *et al.*, 2001; Singh *et al.*, 2003; Mustafa *et al.*, 2005; Jatoi *et al.*, 2007). The rhizome is composed, on a fresh weight basis, of 86% moisture, 0.8% ash, 0.8% total sugars, traces of reducing sugars, 1.4% fibre, 0.1% essential oil, and 6.9% starch and on a dry weight basis, 5.7% ash, 5.8% total sugar, traces of reducing sugars, 10.6% crude fibre, 0.9% essential oil, and 45.6% starch (Policegoudra and Aradhya, 2007). High amylase activity has been reported for *C. amada*; this enzyme converts starch into simple metabolizable sugars, from which, in turn, several valuable aromatic compounds are synthesized (Policegoudra and Aradhya, 2008). Due to this metabolic advantage, the curcumin-free portion is effective in lowering liver cholesterol levels in animals (Srinivasan *et al.*, 2008). Recently, three bioactive terpenoid compounds (difurocumenol, amadannulen, and amadaldehyde) were isolated from mango ginger rhizomes. They also exhibit potential actions such as antimicrobial, antioxidant, platelet aggregation inhibitor activities, and anticancer property (Policegoudra *et al.*, 2010). It also contains antitubercular agents like labdane diterpenoid (Singh *et al.*, 2010).

In plants, secondary metabolites accumulate in specific or specialized cells, tissues, or organs (Flores-Sanchez *et al.*, 2009). *In vitro*, tissues need to undergo dedifferentiation (callus formation) and redifferentiation (rhizogenesis and embryogenesis) processes to achieve the biosynthesis and accumulation of secondary metabolites (Laurain-Mattar *et al.*, 1999; Ramawat and Mathur, 2007). Adventitious root culture, especially cell suspension culture, is a valuable tool for this purpose, and adventitious root induction is the biomass production process most suitable for automation. The present study is a report of a simple and reliable procedure for *in vitro* adventitious root induction from homogenous cell suspension culture of *C. amada* and an examination of the resultant bioactive compounds using gas chromatography-mass spectrometry (GC-MS) analysis.

Results

Initiation of Cell Suspension Culture and Induction of Adventitious Roots

MS medium containing 1.0 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) in combination with 0.25 mg L^{-1} BAP produced friable callus. Medium containing 1.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} BAP was favourable for semi-friable callus formation, and that containing 2.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} BAP was found to produce nonfriable callus (data not shown). To induce adventitious root formation, all three types of calli were transferred to MS liquid medium containing indole-3-butyric acid (IBA) or indole-3-acetic acid (IAA). Friable callus was suspended easily as single cells (Figure 1(a)), and semi-friable callus formed cell aggregation. Nonfriable callus settled down in the medium and could not proliferate into roots (Table 1). The auxins used also significantly influenced adventitious root formation from the callus cultures. The presence of IBA in the medium resulted in a higher percentage of root induction than that of IAA. Maximum root

formation percentage (100%) was obtained from friable callus-derived cell suspension in the medium containing 0.2 and 0.3 mg L^{-1} IBA. Maximum root length (7.23 cm) was observed in the medium containing 0.3 mg L^{-1} IBA (Figure 1(b)). When the concentration of IBA was higher or lower than this level, the percentage of adventitious root formation gradually decreased.

3.2. Optimization of Medium Strength and Sucrose Concentration for Adventitious Root Biomass Production

MS liquid medium strength and sucrose concentration significantly influenced adventitious root formation. Among the tested medium strengths and concentrations of sucrose, the highest root biomass production (51.60 g fresh weight [FW]) was observed in half-strength MS medium supplemented with 3.0% sucrose (Table 2). In contrast, root growth was inhibited when the medium strength or sucrose concentration was higher or lower than this optimum level.

3.3. Optimization of Inoculum Density for Adventitious Root Biomass Production

Inoculum density depends on the volume of culture medium and vessel. In the present study, 250 ml Erlenmeyer flasks containing 50 ml medium were used to determine the optimal inoculum density for maximum root biomass production. M initial inoculum density, maximum adventitious root biomass (121 g FW) and growth rate (12.1%) were recorded at 10 g FW of the initial inoculum (Figure 1(c)). Furthermore, any decrease or increase in inoculum density away from this level led to a decrease in biomass production (Table 3).

3.4. GC-MS Analysis

The essential oil components were found to vary between the rhizomes of field-grown plants and in vitro-raised adventitious roots (Tables 4 and 5). Out of 29 peaks detected in the rhizome samples, 14 were identified in the cultured root samples (Figure 2(a)), and out of 21 peaks detected in the adventitious root samples, 3 were identified in the rhizome samples (Figure 2(b)), with their respective compounds. Interestingly, the in vitro-raised adventitious roots showed only three compounds within the detectable relative percentage zone of the peak area. This was not the case for the rhizome, in which additional compounds were found in detectably larger proportions. Among those three compounds detected in the adventitious root samples, isosorbide and 1-buten-1-ol, 2-methyl-4-(2,6,6,-trimethyl-1-cyclohexen-1-yl)-, formate, (E)- exhibited larger peak areas than in the rhizome samples. The relative peak area of n-hexadecanoic acid was smaller in adventitious root samples than in rhizome samples.

4. Discussion

Adventitious root culture is a valuable biological tool capable of feasible producing bioactive compounds without depending on field-grown parent plants and not subject to outdoor abiotic and biotic factor effects (Sivakumar, 2006; Sivanandhan et al., 2012). In the present study, a promising adventitious root induction system was successfully developed for mango ginger, which is an important aromatic rhizomatous plant. Friable callus responded more favourably in terms of adventitious root formation than semi-friable and nonfriable callus. Prakash et al. (2004) also reported that friable callus seems to be one of the most suitable starting materials for the induction of organogenesis in C. amada. This is probably due to the presence of more physiologically active cells, which are more powerful than the cells in semi-friable and nonfriable callus (Sivanandhan et al., 2012). Our exogenous auxin treatment results indicated that 0.3 mg

L^{-1} IBA was optimum for adventitious root formation, outperforming IAA. Similar phenomena have also been reported in *Withania somnifera* (Sivanandhan *et al.*, 2012), *Morinda citrifolia* (Baque *et al.*, 2010), and *Periploca sepium* (Zhang *et al.*, 2012). Adventitious root culture is not season-dependent and could solve the problem of seasonal availability of mango ginger.

In plant cell/organ culture, sucrose is an important balanced carbon source, acting as a substrate to provide energy for cell growth and thus plays a vital role in the synthesis of cell constituents (Baque *et al.*, 2012). It promotes cell growth via the hydrolysis of invertase, sucrose synthase generates building blocks, and sugars regulate osmotic potential (Stepan-Sarkissian and Fowler, 1986; Calamar and de Klerk, 2002). In the present study, 3% sucrose was suitable for adventitious root growth in terms of biomass production. Lower concentrations did not provide enough energy, and high concentrations negatively affected root primordium induction.

The concentration of salts in the MS medium is an important contributor to biomass production and phytochemical accumulation in cultured cells and tissues (Rajesh *et al.*, 2014). Wu *et al.* (2006) proposed that interactions among nutritional salts enhance the availability of ions to the roots and thereby promote root growth and phytochemical production. In the present study, it was confirmed that the optimization of MS salt concentration is essential for adventitious root production and that half-strength MS medium results in optimal root primordia induction and growth in *C. amada*. The same phenomenon was documented when culturing roots of *Alpinia galanga*, also belonging to the Zingiberaceae family (Rao *et al.*, 2012). Furthermore, we observed that increasing the MS salt strength in the medium, resulted in reduced root biomass production. This suggests that high MS salt concentration produced a stress, thereby reducing the growth of adventitious roots. Determination of the optimal inoculum density is a prerequisite for enhanced production of secondary metabolites from *in vitro*-grown root biomass (Dörnenburg and Knorr, 1995; Jeong *et al.*, 2009; Praveen and Murthy, 2010). In *W. somnifera*, the optimal initial inoculum density is 15 g FW. Higher or lower inoculum densities inhibit root biomass production (Sivanandhan *et al.*, 2012). In the present study, maximum root biomass production in *C. amada* was obtained when the inoculum density was 10 g FW.

In vitro-raised adventitious roots contained higher proportions of two compounds and a similar proportion of a third, compared to the proportions in the field-grown rhizome. This offers a new avenue for scaling up production of the two of the identified compounds, namely isosorbide and n-hexadecanoic acid (Rose and Palkovits, 2012; [ref 34]). Isosorbide, a valuable derivative of glucose, can be used as the chemical basis for the production of green solvents, fuels, fuel additives, and so forth (Rose and Palkovits, 2012). Likewise, n-hexadecanoic acid is a component in the production of cetyl alcohol, which is used in the food and cosmetic industries ([ref 34]). In the present study, the levels of two useful bioactive compounds produced by field-grown plants were successfully reproduced *in vitro*. A study in a related species (*Cucurma longa*) has achieved similar results; this study compared *ex vitro* plants and *in vitro*-raised plants subsequently established *ex vitro* (Singh *et al.*, 2011).

In conclusion, the present investigation opens up a new avenue for the large-scale production of two active compounds, isosorbide and n-hexadecanoic acid, from homogenous cell suspension-mediated adventitious root culture of *C. amada*. To the best of our knowledge, this is the first report of *in vitro* isosorbide and n-hexadecanoic acid production from adventitious root cultures. Furthermore, the results obtained in the present study could be useful in further research on biotransformation and production of these secondary metabolites of *C. amada* on a large scale.

Experimental Procedures

Callus Induction

Microrhizome segments were excised from 3-month-old *in vitro*-grown plants (Raju *et al.*, 2013). For callus induction, these segments were placed on MS medium (Murashige and Skoog, 1962) containing 3.0% sucrose and either concentration of 2,4-D (1.0, 2.0, or 3.0 mg l^{-1}), alone or in combination with BAP or one of Kn (0.25 or 0.5 mg l^{-1}) kinetin. The medium was solidified with 0.8% agar and its pH was adjusted to 5.8 before solidification. The media were autoclaved at 121 °C and 104 kPa for 15 min. Cultures were maintained at a 16 hrs photoperiod under 40 µmol m^{-2} s^{-1} light intensity, provided by white fluorescent tubes, and at a relative humidity of 55–65%.

Initiation of Cell Suspension Culture and Induction of Adventitious Roots

For the induction of adventitious roots, ~250 mg fresh masses of callus (nonfriable, semi-friable, and friable callus) were transferred to separate 150 ml Erlenmeyer flasks containing MS liquid medium. Each flask was supplemented with one of the following concentrations of auxins: 0.1, 0.2, 0.3, 0.4, or 0.5 mg l^{-1} IBA or 0.1, 0.2, 0.3, 0.4, or 0.5 mg l^{-1} IAA; the flasks were then placed on an orbital shaker at 100 rpm in continuous darkness. MS medium without auxin was used as a control. After one week of culture, the proportion of callus responding with root induction (%) was calculated using the following equation:

For biomass production, adventitious roots (~0.5 cm; 35 roots/flask) were transferred, using the same medium composition, cultured, and harvested during the 5th week of culture, when the biomass reached a maximum level. Based on the comparison of root length, the suitable auxin was selected for further studies.

Optimization of Medium Strength, Sucrose Concentration, and Initial Inoculum Density for Adventitious Root Culture

The optimal culture medium for adventitious root biomass production was identified by transferring the initial inoculum (~2.5 g FW adventitious roots) to various strengths of MS liquid medium (1/4, 1/2, 3/4, and full strength) and different concentrations of sucrose (1.0, 3.0, 4.5, and 6.0%). The optimal inoculum density for adventitious root biomass production was identified using various levels (2.5, 5.0, 10.0, 15.0, and 20.0 g FW) of the initial inoculum. Each treatment was carried out three times with seven flasks. The growth ratio was calculated using the following equation (Praveen and Murthy, 2010):

GC-MS Analysis

The adventitious root masses (1.0 g FW) harvested from suspension culture and the rhizomes of field-grown plants were subsequently air-dried for 1 hour and completely ground using pestle and mortar. Extraction was carried out by sonication with methanol (10 ml) until the ground root colour changed into white. After centrifugation at 6,000

rpm 15 min, the upper aqueous layer was collected and filtered through a nylon membrane filter and injected into the GC-MS equipment for analysis.

2.4.1. GC-MS Programme

The GC apparatus used was an Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) column, 30 m × 0.25 mm × 0.25 µm, with a film thickness of 0.25 µm, with a GC Clarus 500 chromatograph (both Perkin Elmer, California, USA). The carrier gas rate was 1 mL min^{-1}, split: 10:1. The mass detector was a Turbomass Gold (Perkin Elmer, California, USA) running Turbomass v5.2 software. Each injected sample was 2.0 µL.

2.4.2. Oven Temperature Programme

The oven temperature programme used was as follows: 110 °C for 2.0 min hold; 10 °C/min^{-1} increase up to 200 °C; immediate further increase up to 280 °C at the rate of 5 °C/min^{-1}; and hold for 9.0 min. The injector temperature was 250 °C, and the total GC running time was 36 min.

2.4.3. MS Programme

The MS conditions were as follows: inlet line temperature, 200 °C; source temperature, 200 °C; electron energy, 70 Ev; mass scan (*m/z*), 45–450; solvent delay, 0–2.0 min; and total MS running time, 36 min. The library used was NIST version 2005.

2.5. Statistical Analysis

All experimental data were subjected to one-way ANOVA followed by statistical significance testing. Data are presented as means ± SE. The mean separations were analysed using Duncan's multiple range test, with $P < [x]$ considered significant (IBM SPSS statistics).

References

1. S. Dutt and J. N. Tayal, "Chemical examination of the essential oil derived from the rhizomes of *Curcuma amada* Roxb," *The Indian oil and soap journal*, vol. 7, pp. 200–205, 1941.
2. A. S. Gholap and C. Bandyopadhyay, "Characterization of mango-like aroma in *Curcuma amada* Roxb," *Journal of Agricultural and Food Chemistry*, vol. 32, no. 1, pp. 57–59, 1984.
3. A. S. Rao, B. Rajanikanth, and R. Seshadri, "Volatile aroma components of *Curcuma amada* Roxb," *Journal of Agricultural and Food Chemistry*, vol. 37, no. 3, pp. 740–743, 1989.
4. S. N. Choudhury, L. C. Rabha, P. B. Kanjilal, A. C. Ghosh, and P. A. Leclercq, "Essential oil of *Curcuma amada* Roxb. from Northeastern India," *Journal of Essential Oil Research*, vol. 8, no. 1, pp. 79–80, 1996.
5. A. K. Srivastava, S. K. Srivastava, and N. C. Shah, "Constituents of the rhizome essential oil of *Curcuma amada* Roxb. from India," *Journal of Essential Oil Research*, vol. 13, no. 1, pp. 63–64, 2001.
6. G. Singh, O. P. Singh, M. P. de Lampasona, and C. Catalan, "*Curcuma amada* Roxb.—chemical composition of rhizome oil," *Indian Perfumer*, vol. 47, pp. 143–146, 2003.
7. A. Mustafa, M. Ali, and N. Z. Khan, "Volatile oil constituents of the fresh rhizomes of *Curcuma amada* Roxb," *Journal of Essential Oil Research*, vol. 17, no. 5, pp. 490–491, 2005.

8. S. A. Jatoi, A. Kikuchi, S. A. Gilani, and K. N. Watanabe, "Phytochemical, pharmacological and ethnobotanical studies in mango ginger (*Curcuma amada* Roxb.; Zingiberaceae)," *Phytotherapy Research*, vol. 21, no. 6, pp. 507–516, 2007.

9. R. S. Policegoudra and S. M. Aradhya, "Biochemical changes and antioxidant activity of mango ginger (*Curcuma amada* Roxb.) rhizomes during postharvest storage at different temperatures," *Postharvest Biology and Technology*, vol. 46, no. 2, pp. 189–194, 2007.

10. R. S. Policegoudra and S. M. Aradhya, "Structure and biochemical properties of starch from an unconventional source-Mango ginger (*Curcuma amada* Roxb.) rhizome," *Food Hydrocolloids*, vol. 22, no. 4, pp. 513–519, 2008.

11. M. R. Srinivasan, N. Chandrasekhara, and K. Srinivasan, "Cholesterol lowering activity of mango ginger (*Curcuma amada* Roxb.) in induced hypercholesterolemic rats," *European Food Research and Technology*, vol. 227, no. 4, pp. 1159–1163, 2008.

12. R. S. Policegoudra, K. Rehna, L. J. Rao, and S. M. Aradhya, "Antimicrobial, antioxidant, cytotoxicity and platelet aggregation inhibitory activity of a novel molecule isolated and characterized from mango ginger (*Curcuma amada* Roxb.) rhizome," *Journal of Biosciences*, vol. 35, no. 2, pp. 231–240, 2010.

13. S. Singh, J. K. Kumar, D. Saikia et al., "A bioactive labdane diterpenoid from *Curcuma amada* and its semisynthetic analogues as antitubercular agents," *European Journal of Medicinal Chemistry*, vol. 45, no. 9, pp. 4379–4382, 2010.

14. I. J. Flores-Sanchez, J. Peč, J. Fei, Y. H. Choi, J. Dušek, and R. Verpoorte, "Elicitation studies in cell suspension cultures of *Cannabis sativa* L.," *Journal of Biotechnology*, vol. 143, no. 2, pp. 157–168, 2009.

15. D. Laurain-Mattar, F. Gillet-Manceau, L. Buchon, S. Nabha, A. Fliniaux M.-, and A. Jacquin-Dubreuil, "Somatic embryogenesis and rhizogenesis of tissue cultures of two genotypes of *Papaver somniferum*: relationships to alkaloid production," *Planta Medica*, vol. 65, no. 2, pp. 167–170, 1999.

16. G. Ramawat and M. Mathur, "Factors affecting the production of secondary metabolites," in *Biotechnology, Secondary Metabolites, Plants and Microbes*, K. G. Ramawat and J. M. Merillo, Eds., pp. 59–102, Science Publishers, Enfield, NH, USA, 2007.

17. C. S. Raju, K. Kathiravan, A. Aslam, and A. Shajahan, "An efficient regeneration system via somatic embryogenesis in mango ginger (*Curcuma amada* Roxb.)," *Plant Cell, Tissue and Organ Culture*, vol. 112, no. 3, pp. 387–393, 2013.

18. T. Murashige and F. Skoog, "A revised medium for rapid growth and bio assays with tobacco tissue cultures," *Physiologia Plantarum*, vol. 15, no. 3, pp. 473–497, 1962.

19. N. Praveen and H. N. Murthy, "Production of withanolide-a from adventitious root cultures of *Withania somnifera*," *Acta Physiologiae Plantarum*, vol. 32, no. 5, pp. 1017–1022, 2010.

20. G. Sivakumar, "Bioreactor technology: a novel industrial tool for high-tech production of bioactive molecules and biopharmaceuticals from plant roots," *Biotechnology Journal*, vol. 1, no. 12, pp. 1419–1427, 2006.

21. G. Sivanandhan, M. Arun, S. Mayavan et al., "Chitosan enhances withanolides production in adventitious root cultures of *Withania somnifera* (L.) Dunal," *Industrial Crops and Products*, vol. 37, no. 1, pp. 124–129, 2012.

22. S. Prakash, R. Elangomathavan, S. Seshadri, K. Kathiravan, and S. Ignacimuthu, "Efficient regeneration of *Curcuma amada* Roxb. plantlets from rhizome and leaf sheath explants," *Plant Cell, Tissue and Organ Culture*, vol. 78, no. 2, pp. 159–165, 2004.

23. M. A. Baque, E. J. Lee, and K. Y. Paek, "Medium salt strength induced changes in growth, physiology and secondary metabolite content in adventitious roots of Morinda citrifolia: the role of antioxidant enzymes and phenylalanine ammonia lyase," *Plant Cell Reports*, vol. 29, no. 7, pp. 685–694, 2010.

24. J. Zhang, W.-Y. Gao, J. Wang, and X.-L. Li, "Effects of sucrose concentration and exogenous hormones on growth and periplocin accumulation in adventitious roots of *Periploca sepium* Bunge," *Acta Physiologiae Plantarum*, vol. 34, pp. 1345–1351, 2012.

25. M. A. Baque, A. Elgirban, E.-J. Lee, and K.-Y. Paek, "Sucrose regulated enhanced induction of anthraquinone, phenolics, flavonoids biosynthesis and activities of antioxidant enzymes in adventitious root suspension cultures of *Morinda citrifolia* (L.)," *Acta Physiologiae Plantarum*, vol. 34, no. 2, pp. 405–415, 2012.

26. A. Calamar and G. J. de Klerk, "Effect of sucrose on adventitious root regeneration in apple," *Plant Cell, Tissue and Organ Culture*, vol. 70, no. 2, pp. 207–212, 2002.

27. G. Stepan-Sarkissian and M. W. Fowler, "The metabolism and utilization of carbohydrates by suspension cultures of plant cells," in *Carbohydrate Metabolism in Cultured Cells*, M. J. Morgan, Ed., pp. 151–182, Springer, Boston, Mass, USA, 1986.

28. M. Rajesh, G. Sivanandhan, M. Arun et al., "Factors influencing podophyllotoxin production in adventitious root culture of *Podophyllum hexandrum* Royle," *Acta Physiologiae Plantarum*, vol. 36, no. 4, pp. 1009–1021, 2014.

29. C. H. Wu, Y. H. Dewir, E. J. Hahn, and K. Y. Paek, "Optimization of culturing conditions for the production of biomass and phenolics from adventitious roots of *Echinacea angustifolia*," *Journal of Plant Biology*, vol. 49, no. 3, pp. 193–199, 2006.

30. K. Rao, B. Chodisetti, L. N. Mangamoori, and A. Giri, "Agrobacterium-mediated transformation in *Alpinia galanga* (Linn.) willd. for enhanced acetoxychavicol acetate production," *Applied Biochemistry and Biotechnology*, vol. 168, no. 2, pp. 339–347, 2012.

31. H. Dörnenburg and D. Knorr, "Strategies for the improvement of secondary metabolite production in plant cell cultures," *Enzyme and Microbial Technology*, vol. 17, no. 8, pp. 674–684, 1995.

32. C. S. Jeong, H. N. Murthy, E. J. Hahn, H. L. Lee, and K. Y. Paek, "Inoculum size and auxin concentration influence the growth of adventitious roots and accumulation of ginsenosides in suspension cultures of ginseng (*Panax ginseng* C.A. Meyer)," *Acta Physiologiae Plantarum*, vol. 31, no. 1, pp. 219–222, 2009.

33. M. Rose and R. Palkovits, "Isosorbide as a renewable platform chemical for versatile applications-quo vadis?" *ChemSusChem*, vol. 5, no. 1, pp. 167–176, 2012.View at:

34. http://en.wikipedia.org/wiki/Palmitic_acid.

35. S. Singh, A. Kuanar, S. Mohanty, E. Subudhi, and S. Nayak, "Evaluation of phytomedicinal yield potential and molecular profiling of micropropagated and conventionally grown turmeric (*Curcuma longa* L.)," *Plant Cell, Tissue and Organ Culture*, vol. 104, no. 2, pp. 263–269, 2011.

Table captions

Table 1: Effect of auxins on *Curcuma amada* adventitious root formation from callus via cell suspension culture.

Table 2: Effect of medium strength and sucrose concentration on *Curcuma amada* adventitious root formation.

Table 3: Effect of the initial inoculum density on *Curcuma amada* adventitious root formation.

Table 4: Phytochemical profile of the field-grown rhizome of *Curcuma amada*.

Table 5: Phytochemical profile of *in vitro*-raised adventitious roots of *Curcuma amada*.

Figure captions

Figure 1: Adventitious root culture of *Curcuma amada* via cell suspension culture. (a) Adventitious root induction from friable callus-derived cell suspension in MS liquid medium supplemented with 0.3 mg l^{-1} indole-3-butyric acid (IBA). (b) Adventitious root growth in MS liquid medium supplemented with 0.3 mg l^{-1} IBA after 5 weeks of culture. (c) Vigorous growth following inoculation with an initial inoculum mass of 10 g FW. Scale bars: (a–c) 0.5 cm.

Figure 2: Gas chromatography-mass spectrometry spectra from methanol extracts of *Curcuma amada*. (a) Field-grown rhizome. (b) Cell suspension adventitious root material derived from friable callus and cultured in liquid half-strength MS medium supplemented with 3.0% sucrose.