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Full Length Research Paper

Micropropagation of *Gynura procumbens* (Lour.) Merr. an important medicinal plant

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A rapid micropropagation protocol was established for *Gynura procumbens* (Lour.) Merr., an important medicinal plant for the treatment of various ailments such as diabetes, hypertension and urinary tract infection. The nodal segments of one year old mature plants was used as the explants for the initiation of axillary branching using Murashige and Skoog (1962) medium (MS) supplemeted with 2 mg l^{-1} BA. Rapid proliferation of shoots was achieved by culturing the *in vitro* shoots derived from the nodal segments onto MS medium supplemented with 2.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA. An average of 18.2 ± 0.6 shoots were produced from each shoot explant. All the micro-shoots produced normal roots within two weeks of culture on the basic MS medium without any plant growth regulators. All the rooted plantlets of *G. procumbens* (100%) survived when they were planted into Jiffy-7 and placed on a shaded place with a temperature of 27 - 30ºC for two weeks. An average of 95 – 98% of the acclimatized plantlets survived after two weeks of transferring into the organic soil: sand mixture (1:1). All the *in vitro* derived plants displayed normal developement as that of the mother plants when they reached mature stage.

Key words: medicinal plant, micropropagation, nodal segment, shoot proliferation.

INTRODUCTION

Gynura procumbens (Lour.) Merr. (Family Asteraceae) is an important medicinal plant indigenous to Malaysia, Indonesia and Thailand. In Malaysia this species has its distribution limited to the western part of peninsular Malaysia. It has several scientific synonym names such as *Gynura sarmentosa*, DC. (Ridley, 1923), *Cacalia procumbens* Lour. (Burkill, 1966) and *Calacia procumbens*, Lour. (Wiart, 2002). It is commonly known as 'sambung nyawa' by the Malays and 'bai bing ca' by the Chinese in Malaysia. Growing approximately 10 – 25 cm tall, this tropical herbaceous medicinal plant, is hightly branched with hairy green leaves that are alternately arranged on hairy purple stem. It produces purple tubular bisexual flowers (Wiart, 2002).

G. procumbens have been long used as ethnoherbal products to threat various ailments such as diabetes, hypertension, urinary infection and used as anti-inflammatery and anti-allergic agents (Jiratchariyakul et al., 2000). However its phytochemical properties have not been well studied until recently. Bohari et al. (2006) reported the extracts of this plant had an enhancing effect on glucose uptake in 3T3 adipocyte cell lines and they suggested that the anti-diabetic action of *G. procumbens* might be mediated through the stimulation of glucose uptake. Iskander et al. (2004) discovered that the crude ethanolic extracts of *G. procumbens* showed anti-inflammatory properties and steroid might be one class of anti-inflammatory compounds found in this plant. Zhang and Tan (2000) reported that the leaves extracts of *G. procumbens* had significantly suppressed the elevated serum glucose levels and reduced the serum cholesterol and triglyceride levels in diabetic rats. Akowuah et al. (2001, 2002) discovered that the n-butanol extracts of this plant could reduce the blood glucose levels in streptozotocin-induced type 2 diabetic rats. Two compounds, 3, 5-di-O-caffeoylquinic acid and 4, 5-di-O-caffeoylquinic acid, identified from this plant were found to inhibit the replication of viruses (Jiratchariyakul et al., 2000).

This plant is conventionally propagated by cuttings. The conventional method cannot meet the increasing demand

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Figure 1. One year old mature plant of *G. Procumbens*

of this plant used as the raw material for the preparation of pharmaceutical, dermaceutical and aromatherapeutical products. The *in vitro* culture techniques can be the alternative for the continuous provision of plantlet stocks for large scale field cultivation. More and more medicinal plant species are now propagated via *in vitro* culture techniques, just to mention a few, such as *Centella asiatica* L. (Tiwari et al., 2000), *Hypericum perforatum* L. (Santarém and Astarita, 2003), *Vitis thunbergii* Sieb. et Zucc. (Mei, 2005) and even woody medicinal plant, *Garcinia indica* (Malik et al., 2005).

We therefore investigated the most suitable micropropagation protocol for the production of *G. procumbens* plantlets en masse. Here, we report an efficient protocol which can be used at a large scale for the clonal multiplication of this plant species using nodal segments as explants derived from the adult plants and followed by the optimized conditions for *in vitro* rooting and further transfer into the greenhouse. To our knowledge, there are no reports on the micropropagation of this plant species.

MATERIALS AND METHODS

The nodal segments, derived from the one year old mature plants (Figure 1) planted at the Universiti Sains Malaysia campus, Penang, Malaysia, were used as initial explants for the establishment of *in vitro* culture system for mass production of *G. procumbens* plantlets. The nodal segments of about 1.0 cm in length were washed with detergent and rinsed under running tap water for 30 min. They were immersed in 95% ethanol for 30 s and then surfaced sterilized with 20% Clorox® containing 5.3% sodium hypochlorite for 15 min, and rinsed three times with sterile distilled water. They were again surface sterilized second time with 5% Clorox® and rinsed again three times with sterile distilled water. Both ends of the nodal segments were cut to obtain 0.5 – 0.6 cm nodal segment explants and were inoculated onto MS (Murashige and Skoog, 1962) medium containing 30 g l^{-1} sucrose and 7.5 g l^{-1} agar (Algas, Chile). After 10 days, the percentage of aseptic nodal segments obtained was determined.

The aseptic nodal segments were used for the establishment of best shoot proliferation medium by transferring them onto MS medium supplemented with 0 – 10 mg l^{-1} benzyladenine (BA), 0 – 10 mg l^{-1} 1-naphthaleneacetic acid (NAA), 30 g l^{-1} sucrose and 7.5 g l^{-1} agar. The study was carried out using 6 x 6 factorial design and number of shoots formed from each nodal segment was determined after four weeks of culture. The data collected was analyzed using 2-way analysis of variance (ANOVA) followed by Duncan Multiple Range test at p = 0.05 to determine the best plant growth regulator combination for the production of multiple shoots.

Shoot explants derived from the branched shoots initiated from the nodal segments cultured on the initial BA and NAA supplemented MS medium were further subcultured on MS medium supplemented with 0 – 3 mg l^{-1} BA and 0.5 mg l^{-1} NAA or without NAA to investigate further whether low concentration of BA and NAA could be used for the induction of multiple shoot formation of *G. procumbens*. The study was carried out using 2 x 4 factorial design. The number of shoots produced from each explant was determined after four weeks of culture. The data was analyzed with 2-way ANOVA and the best proliferation medium was determined using Duncan Multiple Range test at p = 0.05.

For both of these studies, approximately 20 ml of medium was dispensed in 100 ml glass jars. Three explants were cultured in each glass jar (experimental unit) and ten units were used for each combination medium.

To determine whether liquid medium could accelerate the formation of multiple shoot, the nodal segments were cultured into 100 ml Erlenmeyer flasks containing MS supplemented with 2 mg l^{-1} BA, shoot proliferation medium. The shoot morphology and the number of shoots formed after four weeks of culture was observed and determined respectively.

The effect of auxin on efficiency of *in vitro* rooting was examined. The micro-shoots were separated from the multiple shoots and were cultured into test tubes containing 15 ml of MS medium supplemented with 0 – 10 mg l^{-1} NAA. Percentage of shoots produced roots and the number of roots formed were determined every week for five weeks after culture.

All the *in vitro* cultures were placed in the culture room maintained at 25 ± 2ºC under continuous photoperiod with a photosynthetic photon flux density (PPFD) of 45 µmol m^{-2} s^{-1} provided by cool white fluorescent lamps (Philips Electronics N.V. Holland). The pH of all the media was adjusted to 5.7 – 5.8 before autoclaving at 121°C for 13 min under a pressure of 1.05 kg cm^{-2} (Tommy Autoclave SS-325).

To establish the acclimatization protocol, the rooted shoots (plantlets) were removed from the gelled rooting medium and washed under running water to remove traces of medium. The plantlets were planted into Jiffy-7 and placed into plastic trays covered with transparent plastic sheets punched with a few openings. The number of openings was increased daily until the plastic sheet was totally removed after one week. The trays were placed on the shaded corridor with a temperature of 27 - 30ºC for two weeks. Percentage of plantlets survived after two weeks of transferring into the organic soil: sand mixture (1:1) was determined.

RESULTS AND DISCUSSION

High aseptic nodal segments of *G. procumbens* (90 ± 5%) were successfully established on full strength MS medium and the remainder of the contaminated cultures were mainly due to fungal contamination. Emergence of 2 - 3 leaves from each nodal segment could be noticed after 10 days in this plant growth regulator (PGR) free MS

Table 1. Effect of BA and NAA (0 – 10 mg l^{-1}) supplemented into MS medium on induction of multiple shoots formation from the Nodal Segments of *G. procumbens* after 4 weeks of culture

Medium MS + BA (mg l^{-1}) + NAA (mg l^{-1})	Mean no. of shoot /nodal segment ± s.e
NAA = 0.0; BA= 0.0	± 0.0 a
2.0	12.3 ± 1.1 e
4.0	± 1.3 d
6.0	± 1.7 d
8.0	8.0 ± 1.6 d
10.0	7.3 ± 1.7 d
NAA = 2.0; BA= 0.0	± 0.0 a
2.0	3.8 ± 0.4 c
4.0	± 0.6 b
6.0	3.6 ± 0.9 bc
8.0	3.1 ± 1.1 ab
10.0	2.5 ± 0.3 a
NAA = 4.0; BA= 0.0	± 0.0 a
2.0	2.7 ± 0.4 ab
4.0	1.9 ± 0.3 a
6.0	2.6 ± 0.5 a
8.0	2.3 ± 0.7 a
10.0	1.9 ± 0.3 a
NAA = 6.0; BA= 0.0	± 0.0 a
2.0	1.8 ± 0.6 a
4.0	2.2 ± 0.5 a
6.0	1.4 ± 0.4 a
8.0	1.3 ± 0.3 a
10.0	1.3 ± 0.1 a
NAA = 8.0; BA= 0.0	± 0.0 a
2.0	1.3 ± 0.3 a
4.0	1.9 ± 0.4 a
6.0	1.2 ± 0.2 a
8.0	1.5 ± 0.3 a
10.0	1.4 ± 0.2 a
NAA = 10.0; BA= 0.0	± 0.0 a
2.0	1.8 ± 0.5 a
4.0	1.8 ± 0.6 a
6.0	1.8 ± 0.4 a
8.0	1.4 ± 0.3 a
10.0	1.0 ± 0.0 a

medium. The use of simple salt plus sucrose medium is adopted as the usual method to reveal and eliminate the contamination in initiation culture stage in many laboratoties (George, 1993).

MS medium supplemented with BA and NAA induced the formation of multiple shoots from the nodal segments of *G. procumbens.* An average of 7 to 12 shoots were formed from each nodal segment when they were cultured on MS medium supplemented with 2 – 10 mg l^{-1} BA without the addition of NAA. MS medium supplemented with 2 mg l^{-1} BA induced the most number of shoots from the nodal segments, 12.3 ± 1.1 shoots per explant (Table 1). These shoots appeared to proliferate from the node via axillary branching of buds from the explants (Figure 2). The addition of NAA, as little as 2 mg l^{-1}, into the MS medium with the presence of BA had reduced tremendously the formation of multiple shoot. No multiple shoots was observed in control (PGR free medium) and on all MS media that were only supplemented with NAA (Table 1). Similar results were reported in *C. asiatica* (George et al., 2004; Raghu et al., 2007), *Hemidesmus indicus* (Patnaik and Debata, 1996) and *Vitex negundo* L. (Sahoo and Chand, 1998; Usha et al., 2007).

These results indicated that BA, a cytokinin, played an important role in induction of multiple shoot formation and was very effective in shoot proliferation. However, BA at higher concentrations not only reduced the number of shoots formed but also resulted in stunted growth of the shoots. When the shoot explants of these *in vitro* multiple shoot cultures were transferred onto MS supplemented 1 - 3 mg l^{-1} BA with or without the presence of 0.5 mg l^{-1} NAA, more shoots were produced with the presence of

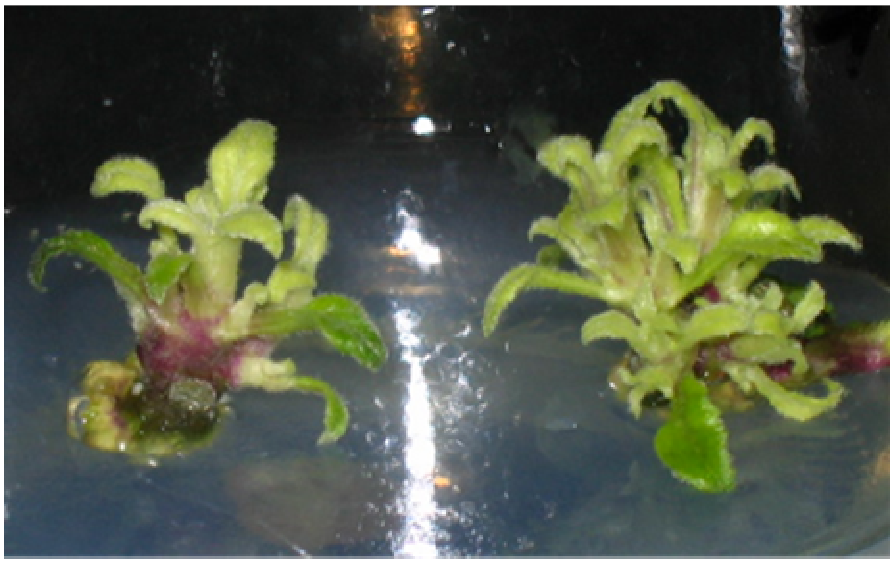

Figure 2. Axillary branching of buds from the nodal segments of *G. procumbens* after 4 weeks of culture on MS medium supplemented with 2 mg l^{-1} BA

Table 2. Effect of BA (0 – 3 mg l^{-1}) and NAA (0, 0.5 mg l^{-1}) suplemented into MS medium on multiple shoot formation from the shoot explants of *G. procumbens* after 4 weeks of culture.

Medium MS + BA (mg l^{-1}) + NAA (mg l^{-1})	Mean no. of shoot /shoot explant ± s.e
NAA = 0.0; BA= 0.0	± 0.0 a
1.0	8.9 ± 1.5 b
2.0	13.3 ± 2.1 c
3.0	10.4 ± 1.3 bc
NAA = 0.5; BA= 0.0	± 0.0 a
1.0	9.8 ± 0.4 b
2.0	18.2 ± 0.6 d
3.0	13.6 ± 0.9 c

0.5 mg l^{-1} NAA in the BA supplemented MS medium (Table 2). The result showed that the presence of low concentrations of an auxin (NAA) in combination with a cytokinin (BA) positively enhanced the frequency of shoot induction and growth. This indicated the synergistic effect of a cytokinin (BA) and an auxin (NAA). MS medium supplemented with 2.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA was found to be the most effective medium for shoot multiplication resulting in the formation of an average of 18.2 ± 0.6 shoots per explant. Similar synergistic effects were demonstrated for many plants propagated *in vitro*, for example *Santolina canescens* (Casado et al., 2002), *Curcuma zedoaria* Roscoe and *Zingiber zerumbet* Smith (Christine and Chan, 2007), Rotula aquatica (Martin, 2003), and turmeric (Salvi et al., 2002).

When the nodal segments were cultured in the liquid shoot proliferation medium (MS + 2 mg l^{-1} BA + 0.5 mg l^{-1} NAA), only 50% of the nodal segments produced multiple shoots of 2 – 3 shoots per nodal segment while the others remained as single shoot after 4 weeks of culture. All the shoots became vetrified as early as three weeks in the liquid culture medium (Figure 3).

Results (Figure 4) showed that basic MS medium without the addition of any NAA was sufficient for the establishment of *in vitro* rooting of the micro-shoots of *G. procumbens.* Percentage of micro-shoots produced roots became increasingly reduced with increased concentration of NAA supplemented into the MS medium. Hence, gelled basic MS medium was determined as the suitable medium for *in vitro* rootng of *G. procumbens.* All the micro-shoots produced normal roots within two weeks of culture in the basic MS medium (Figure 5). Some plant species can root easily without any plant growth regulators while some need the assistance of auxin for successful *in vitro* rooting of micro-shoots. Besides *G. procumbens,* rooting can proceed without any plant

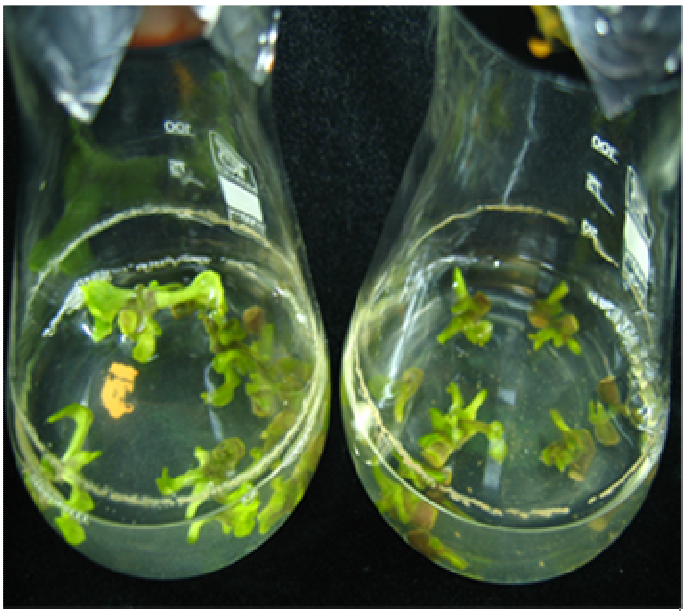

Figure 3. Shoots derived from the nodal segments of *G. procumbens* became vetrified in liquid MS medium supplemented with 2 mg l^{-1} BA + 0.5 mg l^{-1} NAA after 3 weeks of culture

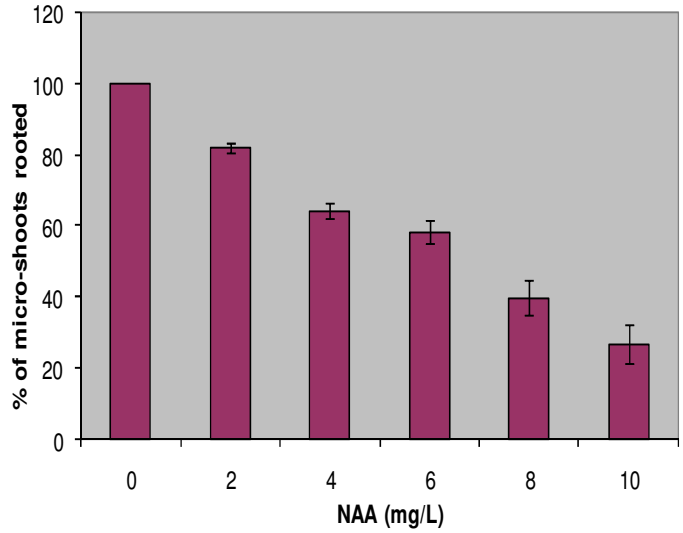

Figure 4. Effect of NAA on *in vitro* rooting of *G. Procumbens* micro-shoots

growth regulators as in *Salvia miltiorrhiza* (Morimoto et al., 1994); *Salvia bancoana* and *Salvia valentine* (Cuenca and Amo-Marco, 2000). On the other hand, Arikat et al. (2004) and Miŝić et al. (2006) reported that auxin was needed for the rooting of *Salvia fruticosa* and *Salvia brachyodon* respectively.

All the rooted plantlets of *G. procumbens* (100%) survived when they were planted into Jiffy-7 and placed on the shaded corridor with a temperature of 27 - 30ºC for two weeks (Figure 6A). An average of 95 – 98% of the

Figure 5. *In vitro* rooting of *G. procumbens* micro-shoots in gelled MS medium after 2 weeks of culture

acclimatizd plantlets survived after two weeks of transferring into the orgaic soil: sand mixture (1:1) (Figure 6B). We observed that the addition of sand had increased the survival rate of the acclimatized planlets. The presence of sands had reduced the retention of water common-ly occurred in the organic soil which caused rotting of the roots and eventually the whole plantlets. Six months later all the *in vitro* derived plants displayed normal development as that of the mother plants (Figure 6C).

In conclusion, the micropropagation protocol reproted here was characterized with a rapid proliferation of shoots, easy rooting of the micro-shoots and the plantlets were easily acclimatized to the external environment and undergoing normal physiological developement. This is highly advantageous for the production of uiniform source of *G. procumbens* plants for a range of further biotechnological applications.

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REFERENCES

Akowuah GA, Sadikun A, Mariam A (2002). Flavonoid Identification and Hypoglycaemic Studies of the Butanol Fraction from Gynura procumbens. Pharm Biol. 40(6):405-410.

Akowuah GA, Sadikun A, Mariam A, Aminah I (2001). Blood Sugar Lowering Activity of *Gynura procumbens* Leaf Extracts. J Trop. Med. Plants. 2(1):5-10.

Arikat NA, Jawad FM, Karam NS, Shibli RA (2004). Micropropagation and accumulation of essential oils in wild sage (Salvia fruticosa Mill.). Scientia Horticulturae. 100:193–202.

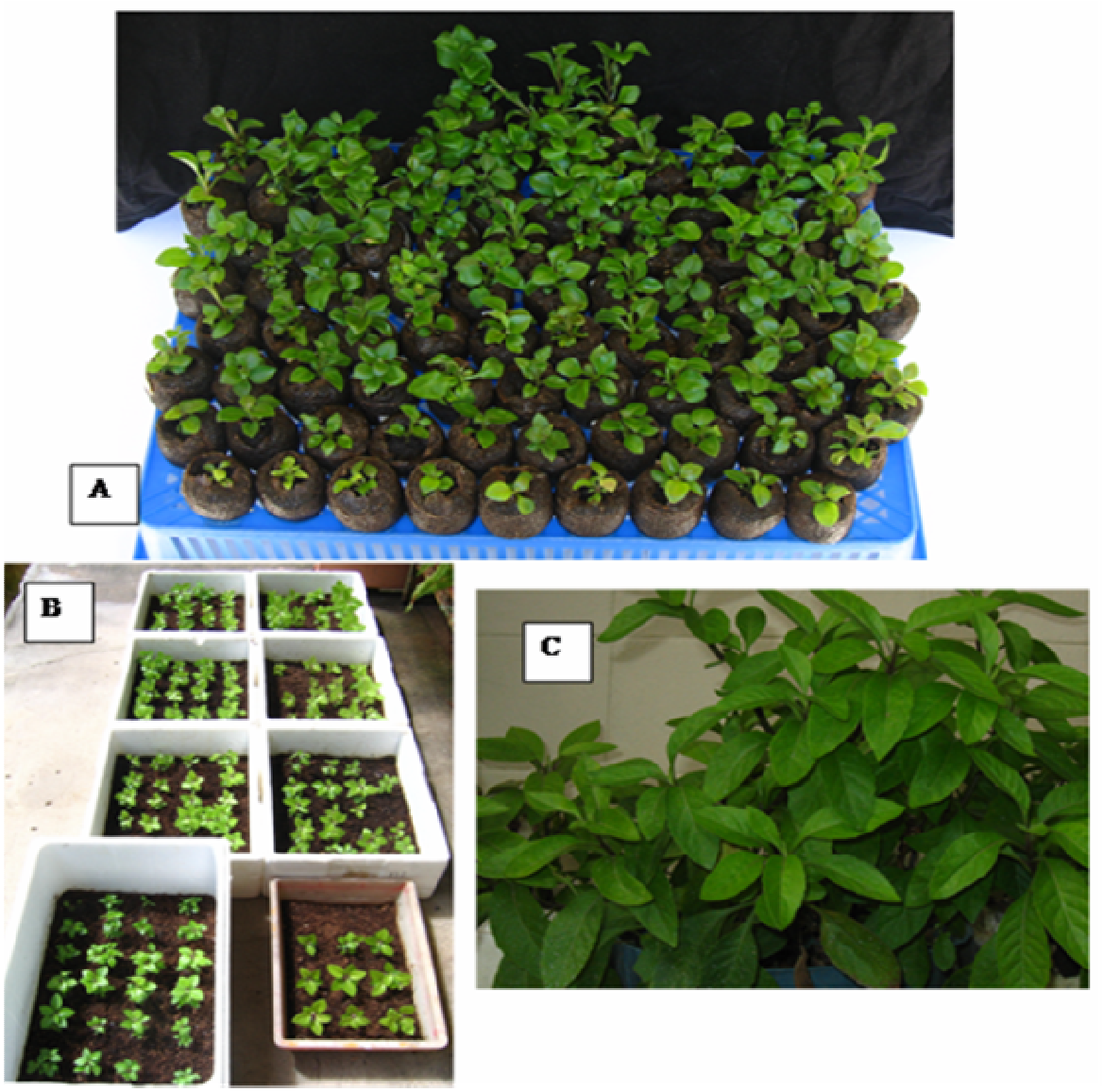

Figure 6. Rooted *in vitro* plantlets of *G. procumbens* planted in Jiffy-7 (A); Two weeks old acclimatized *Q. procumbens* plantlets in organic soil: sand mixture (1:1) (B); 6months old *in vitro* derived plants (C).

Bohari M, Pauliena S, Muhajir H, Khozirah S, Lajis N (2006). Glucose uptake: *s*timulatory Activity of Gynura procumbens in 3T3- F442A adipocytes. In: Malaysian Medicinal Plant: Chemistry and Biological Activity. UNIMAS and Malaysian Natural Products Society, Sarawak.

Burkill IH (1966). A Dictionary of the Economic Products of the Malay Peninsula, Vol.1 (A-H). Publication Unit Ministry of Agriculture Malaysia, Kuala Lumpur, Malaysia.

Casado JP, Navarro MC, Utrilla MP, Martinez A, Jimenez J (2002). Micropropagation of *Santolina canescens* Lagasca and *in vitro* volatiles production by shoot explants. Plant Cell Tiss Org. 69:147–153.

Christine S, Chan LK (2007). Micropropagation of *Curcuma zedoaria* Roscoe and *Zingiber zerumbet* Smith. Biotechnol. 6(4): 555-560.

Cuenca S, Amo-Marco JB (2000). *In vitro* propagation of two Spanish endemic species of Salvia through bud proliferation. In Vitro Cell Dev-Pl. 36:225–229.

George EF (1993). Plant Propagation by Tissue Culture. 2nd Edn., Exegetics Limited, England.

George S, Ramashree AB, Sabastian D, Hariharan M (2004). Micropropagation of *Centela asiatica* L. through axillary bud multiplication. Phytomorphol. 54: 31-34.

Iskander MN, Song Y, Coupar IM, Jiratchariyakul W (2004). Antiinflammatory screening of the medicinal plant *Gynura procumbens.* Plant Food Hum Nutr. 57(3-4): 233 – 244.

Jiratchariyakul W, Jarikasem S, Siritantikorn S, Somanabandhu A, Frahm AW (2000). Antiherpes Simplex Viral Compounds from *Gynura procumbens* Merr. Mahidol University Annual Research Abstracts 28: 182.

Malik SK, Chaudhury R, Kalia RK (2005). Rapid *in vitro* multiplication and conservation of *Garcinia indica*: A tropical medicinal tree species. Scientia Horticulturae. 106:539-553.

Martin KP (2003) Rapid in vitro multiplication and ex vitro rooting of Rotula aquatica Lour., a rare rhoeophytic woody medicinal plant. Plant Cell Rep. 21:415–420

Mei CL (2005) Micropropagation of *Vitis thunbergii* Sieb. et. Zucc., a medicinal herb, through high frequency shoot tip culture. Scientia Horticulturae 107:64-69

Mis˘ic´ D, Grubis˘ic´ D, Konjevic´ R (2006) Micropropagation of *Salvia*

brachyodon through nodal explants. Biol. Plantarum 50:473–476
Morimoto S, Goto Y, Shoyama Y (1994) Production of lithospermic acid B and rosmarinic acid in callus tissue and regenerated plantlets of Salvia miltiorrhiza. J. Nat Prod. 57:817–823
Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plantarum 15:473–497
Patnaik J, Debata BK (1996) Micropropagation of *Hemidesmus indicus* R.Br. through axillary bud culture. Plant Cell rep. 15: 427-430
Raghu AV, Martin G, Priya V, Geetha SP, Balachandran I (2007) Low cost alternatives for the micropropagation of *Centella asiatica*. J. Plant Sci. 2(6): 592-599
Ridley HN (1923) The Flora of the Malay Peninsula, Vol II. L. Reeve & Co., Ltd., London
Sahoo Y, Chand PK (1998) Micropropagation of *Vitex negundo* L. a woody aromatic medicinal shrub, through high frequency axillary shoot proliferation. Plant Cell Rep. 18: 301-307
Salvi ND, George L, Eapen S (2002). Micropropagation and field evaluation of micropropagated plants of turmeric. Plant Cell Tiss Org. 68:143–151
Santerém ER, Astarita LV (2003) Multiple shoot formation in *Hypericum perforatum* L. and hypericin production. Brazilian J Plant Physiol. 15(1):43-47
Tiwari KN, Sharma NS, Tiwari K, Singh BD (2000) Micropropagation of *Centella asiatica* (L.), a valuable medicinal herb. Plant Cell Tiss Org. 63(3):179-185
Usha PK, Binjamin S, Mohanan KV, Raghu AV (2007). An efficient micropropagation system for *Vitex negundo* L. an important woody aromatic medicinal plant, through shoot tip culture. Res J Bot. 2: 102 – 1-7
Wiart C (2002). Medicinal Plants of Southeast Asia. Prentice Hall, Malaysia.
Zhang XF, Tan BKH (2000). Effect of an ethanolic extract of *Gynura procumbens* on serum glucose, cholesterol and triglyceride levels in normal and streptozotocin-induced diabetic rats. Singapore Med J. 41(1):9-13.