Journal of Research in Biology

An International Scientific Research Journal

ISSN No: Print: 2231 –6280; Online: 2231- 6299

Original Research

Efficient *in vitro* micropropagation of *Gynura procumbens* - an important rare medicinal plant, through shoot tip and nodal segment explants

Authors:
Farhana Parvin[1], Jikrul Islam Md[1], Naoshin Jahan[1], Habiba Khan[1], Pallob Ebna Shaekh Md[1], Aminul Islam Md[1], Muhammed Hamidur Rahaman[1] and Motiur Rahman Md[2*].

Institution:
1. Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi 6205 Bangladesh.

2. Assistant Professor, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi 6205 Bangladesh.

Corresponding author:
Motiur Rahman Md

Email Id:
rainbristy27@gmail.com

ABSTRACT:

Gynura procumbens is a medicinally important herbaceous plant species belonging to the family Asteraceae. It works against virus, inflammation and various types of allergies. It is used to treat rheumatic fever, migraine, kidney disease, diabetes, dysentery, various types of skin diseases and cancers. This study aimed to develop a suitable protocol for rapid production of *Gynura procumbens* from different explants. Shoot tip and nodal segment explants were used from one year mature plant. For shoot proliferation, among the two explants, shoot tips showed the best response (90%) on Murashige and Skoog (MS) medium supplemented with 1.0mg/l BAP and produced an average of 20±0.8 shootlets in each explants. *In vitro* derived shoots were subcultured on the similar medium and it gave similar production with healthy shoots. 100% rooting was observed on full strength MS medium containing NAA (0.5mg/l). Rooted plantlets were transferred for hardening into the mixture of soil, cowdung and sand (1:1:1). Then the rooted plantlets were successfully established in the field.

Keywords:
Gynura procumbens, *in vitro*, micropropagation, cytokinin, auxin.

Article Citation:
Farhana Parvin, Jikrul Islam, Naoshin Jahan, Habiba Khan, Pallob Ebna Shaekh, Aminul Islam, Muhammed Hamidur Rahaman and Motiur Rahman.
Efficient *in vitro* micropropagation of *Gynura procumbens*- an important rare medicinal plant, through shoot tip and nodal segment explants
Journal of Research in Biology (2014) 4(6): 1444-1450

Web Address:
http://jresearchbiology.com/documents/RA0464.pdf

Dates:
Received: 21 Jul 2014 **Accepted:** 07 Aug 2014 **Published:** 10 Sep 2014

INTRODUCTION

Plants have been an important source of medicine for thousands of years. According to World Health Organisation, about 80% of the population living in developing countries still use traditional medicines derived from plants for their primary health care needs. At present, despite the necessity of synthetic drugs and antibiotics for medical practices, a major contribution is provided by plants to the pharmaceutical industries (Sahoo *et al.,* 1997). *In vitro* micropropagation techniques of important medicinal plants are improving day by day that affects the production of high quality plant-based medicines. Moreover, steady supply of raw materials can be maintained.

Gynura procumbens is considered as an important medicinal plant in South East Asia. It is found to be present throughout the Malay Peninsula along the west side. It is a perennial herb belonging to the family Asteraceae. Its habit is a scrambling or weakly climbing herb with stem up to 6m long. Stems are light purple with green spot. Leaves with green color are appeared at the stalk on stem. The flowers are purple, tubular and bisexual (Wiart, 2002). This plant is maintained as a low bush by trimming and pinching.

Gynura procumbens is commonly known as 'sambung nyawa' that means 'Continuation of Life'. This plant is also known by different names in different parts of the world. This is not a native plant of Bangladesh. The corresponding author collected this experimental plant from a relative who planted this plant in his home garden in Joypurhat, Bangladesh and said that it is a diabetes plant. According to the report of Jiratchariyakul *et al.,* (2000), this plant is useful for the treatment of many ailments like urinary infection, hypertension, diabetes, and anti-allergic agents. They also reported that the replication of viruses could be inhibited by the compounds of this plant. Agustina *et al.,* (2006) discovered that the leaf extract of *Gynura procumbens* has anticarcinogenic effects. Besides this, it also has anti ulcerogenic activity (Mahmood *et al.,* 2010). Several studies have been shown that the leaf extract possess anti hyperglycemic (Li *et al.,* 2009), anti-oxidative (Puangpronpitag *et al.,* 2010), anti-lipidemic (Zhang and Tan, 2000) and anti-inflammatory (Iskander *et al.,* 2002) effects. The leaves of this plant do not have any toxic effects (Rosidah *et al.,* 2009). Akowuah *et al.,* (2001) reported that the extract of *G. procumbens* can reduce the blood sugar level of type 2 diabetic rats. Glucose uptake in 3T3 adipocyte cell lines are affected by the extract of *G. procumbens* (Bohari *et al.,* 2006). They also suggested that the stimulation of glucose uptake might be mediated by the anti-diabetic action of *G. procumbens*.

Medicinal plants are of great interest to the researchers in the field of biotechnology and many other sectors. The propagation method of this plant is cuttings which cannot give sufficient raw materials to produce various types of pharmaceutical, dermaceutical and aromatherapeutcal products. Micropropagation technique can produce a large scale of raw materials and fulfill the demand.

The present study was undertaken to establish a reliable plantlet regeneration protocol using shoot tip and nodal explants for large-scale production of *Gynura procumbens*. To our knowledge there is no report on *in vitro* propagation of *Gynura procumbens* through shoot tip explants.

MATERIALS AND METHODS

Shoot tips and nodal segments of *Gynura procumbens* were collected from garden grown plants. The explants were washed thoroughly in the running tap water for 30 min, followed by treatment with a solution of tween-80 for 10 min and thereafter washed tree times with sterile distilled water. Then the explants were washed with 70% ethanol, 0.1% $HgCl_2$ for 6 min and rinsed with sterile distilled water for four times. The shoot tip and nodal segment were trimmed at both ends

(1-1.5cm) prior to the inoculation on culture media.

Throughout the experiments, full strength MS medium (Murashige and Skoog, 1962) with 3% (w/v) sucrose and 0.75% (w/v) agar were used. The pH of all media were adjusted to 5.7 prior to autoclaving (20 min). The cultures were incubated in a culture room with 25±2°C and 16h photoperiod was provided by cool white fluorescent tubes for four weeks. The basal medium was supplemented with BAP (1-3mg/l) alone and in combination with NAA (0.1-0.5mg/l) or IBA (0.1-0.5mg/l). For further elongation and multiplication of regenerated shoots, the primary shoots were separated aseptically and subcultured.

The microshoots were separated from the multiple shoots and cultured into MS medium supplemented with NAA (0.1-1.5mg/l) or IBA (0.1-1.5mg/l). Plantlets with developed roots were removed from the culture media. By washing through running tap water, roots were transferred to plastic pots containing autoclaved garden soil, cowdung and sand in the ratio of 1:1:1 covered with porous polythene for maintaining high relative humidity (80-90%). After 15 days the plantlets were subsequently transferred to larger pots & gradually acclimatized to outdoor condition

RESULTS AND DISCUSSION

During this study, shoot tips (Figure 1-A, B) and nodal segments (Figure 2-A, B) were cultured on MS medium supplemented with different concentrations of BAP (0.1-3mg/l) alone or in combination with NAA (0.2- 0.5mg/l) or IBA (0.2- 0.5mg/l). Comparing the two explants, the maximum number of multiple shoot proliferation was observed from shoot tip explants in MS medium containing BAP 1.0mg/l, showed better response (90%). On the other hand, in case of nodal explants, 80% response was observed in the similar medium. An average of 20±0.8 shootlets per explants of shoot tip and 15±1.09 of nodal explants was produced after 30 days of culture (Table 1). The average shootlet length was 8±0.71 and 8±1.05 respectively (Table 1).

Table 1: Effect of different concentrations of Cytokinin alone or in combination with auxin in MS media for shoot proliferation from shoot tips and nodal segments of *Gynura procumbens*

	Shoot tips			Nodal segments		
BAP	**Average Number of shoots/explants**	**% of explants responded**	**Mean shoot length (cm)**	**Average number of shoots/explants**	**% of explants responded**	**Mean shoot length (cm)**
0.50	09± 0.13	60	07±0.18	04±0.04	70	6.5±0.33
1.00	**20±0.8**	**90**	**08±0.71**	**15±1.09**	**80**	**08±1.05**
1.50	10±0.09	80	07±0.02	09±0.30	70	06±0.59
2.00	08±0.25	70	6.5±0.18	07±0.19	80	05±.0.33
2.50	05±0.66	50	05±0.35	06±1.01	50	4.5±0.63
3.00	04±0.1	40	3.5±0.11	05±0.19	30	2.7±0.09
BAP+NAA						
0.5+0.2	06±0.17	50	4.5±0.17	05±0.55	40	03±0.4
0.5+0.5	07±0.05	60	05±0.09	07±0.4	50	3.5±0.33
1.0+0.2	09±0.13	70	5.5±0.42	08±0.60	60	4.2±0.8
1.0+0.5	**15±1.13**	**80**	**6.2±0.99**	**12±0.95**	**70**	**06±1.1**
2.0+0.5	06±0.56	60	05±0.02	06±0.09	60	4.5±0.5
3.0+0.5	04±0.23	40	3.2±0.15	03±0.15	30	2.3±0.4
BAP+IBA						
0.5+0.2	03±0.09	50	3.5±0.3	02±0.19	40	2.5±0.16
0.5+0.5	04±0.4	60	05±0.23	04±0.4	50	05±0.5
1.0+0.2	**06±0.85**	**75**	**06±0.9**	**05±0.8**	**70**	**06±0.75**
1.0+0.5	05±0.35	70	05±0.09	04±0.08	60	4.5±0.15
2.0+0.5	04±0.56	60	4.5±0.3	03±0.13	50	3.8±0.25
3.0+0.5	02±0.09	40	2.8±0.29	01±0.23	30	1.5±0.3

Each value represents an average of 10 replicates and each experiment was repeated at least thrice; values are expressed as Mean ± Standard Error.

Figure 1: Direct shoot regeneration from shoot tip in medium having MS + 1.0 mg/l BAP

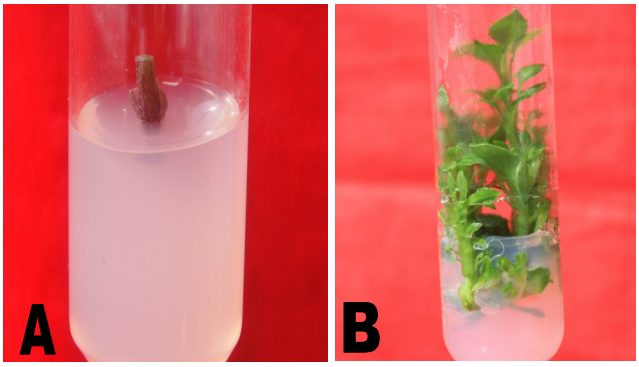

Figure 2: Direct shoot regeneration from nodal segment in medium having MS + 1.0 mg/l BAP.

A study by Verma and Kant (1999) brought the similar results in *Emblica officinalis*. Similar observation was also reported by Deka *et al.,* (1999) in *Withania somnifera* (L). Patnaik and Debata (1996) and Islam *et al.,* (2013, 2014) reported that BAP was superior for multiple shoot induction than other cytokinins in shoot tip explants.

Individual shoots from multiple shoot complexes were separated after 28 days of culture and transferred to full strength of MS medium supplemented with NAA (0.1-1.5mg/l) or IBA 0.1-1.5mg/l). The maximum rooting response (100%) (Figure 3: A) was achieved on medium supplemented with NAA (0.5mg/l) with an average of 15±1.1 roots per shoot explants (Table 2) and the average root length was 7.15±0.9. It is similar to *Centella asiatica* (George *et al.,* 2004; Raghu *et al.,* 2007), *Hemidesmus indicus* (Patnaik and Debata, 1996) and *Vitex negundo L.* (Usha *et al.,* 2007).

Initially, rooted plantlets were gently removed from the test tubes and thoroughly washed with running tap water to remove traces of medium and transferred to plastic pots having vermiculite and soil (1:1) (Figure 2B). After that the pots were kept for a week in a culture room with 25±2°C and 16h photoperiod. The plastic pots were covered in a polyethylene tent to provide sufficient light and moisturizer. The polyethylene covers were

Table 2: Effect of different concentration and combination of auxin on rooting.

Treatment (mg/l)	Days of root initiation	% of root induction	Mean Number of root per explants	Mean length of longest root in cm
NAA				
0.1	18-20	50	5±0.17	4.5±0.26
0.2	10-12	60	8±0.38	5.2±0.47
0.5	**8-9**	**100**	**15±1.1**	**7.15±0.9**
1.0	10-11	80	12±0.4	6.1±0.6
1.5	15-20	70	6±0.27	4.23±0.14
IBA				
0.1	21-23	30	03±0.66	3.2±0.39
0.2	14-15	50	07±0.05	5.5±0.15
0.5	**10-12**	**80**	**11±1.23**	**07±1.1**
1.0	12-13	60	09±0.54	06±0.18
1.5	17-20	50	06±0.25	05±0.2

Each value represents an average of 10 replicates and each experiment was repeated at least thrice, Values are Mean ± Standard Error.

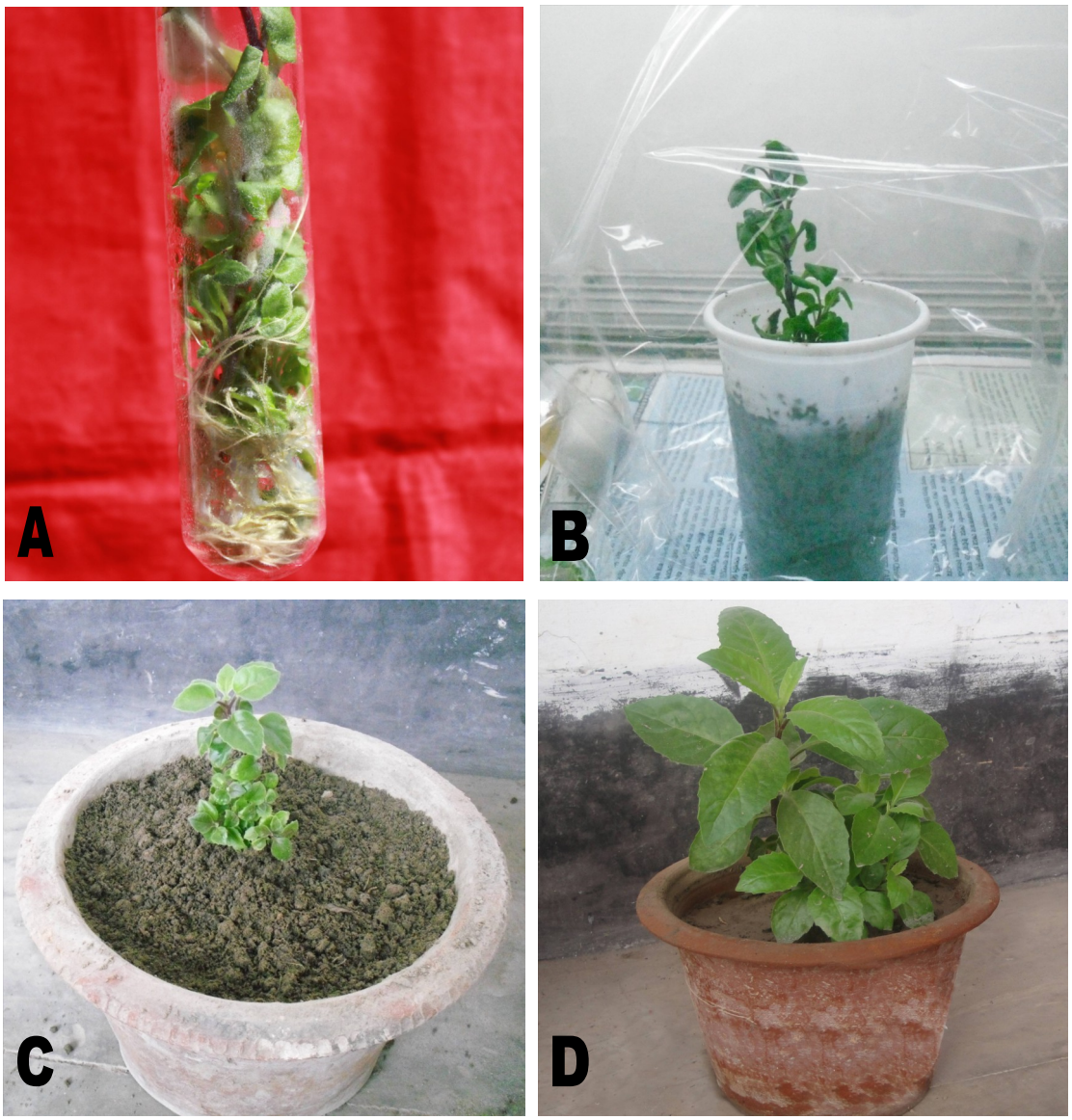

Figure 3:(A) Root induction in medium having MS + 0.5 mg/l NAA.(B) Hardening of *in vitro* cultured plant. (C) *In vitro* cultured plant under natural condition after 10 days of transplantation. (D) *In vitro* cultured plant under natural condition after 2 months of transplantation.

withdrawn after 15 days of hardening. Then the plants were transferred to larger pots filled with soil containing organic manure (Figure 3:C) for further growth. About 100% of plantlets survival was observed after hardening of the regarded *G. procumbens*.

CONCLUSION

In a nutshell, it can be said from the above study that we develop a rapid and efficient protocol for micropropagation from the different explants of *Gynura procumbens*. The results showed the ability of shoot tip and nodal explants to produce shootlets without callus production. So it can be used for the large scale production of this medicinal plant within a short time.

REFERENCES

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.

Agustina D, Haryana SM, Supartinah A. 2006. Anticarcinogenesis effect of *Gynura procumbens* (Lour) merr on tongue carcinogenesis in 4NQO-induced rat. Dental Journal (Maj. Ked. Gigi) 39(3):126- 132.

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

Deka AC, Kalita MC, Baruah A. 1999. *In Vitro* Micropropagation of potent herbal medicinal plant, *Withania somnifera* (Ashwagandha). Environmental Ecology. 17(3): 594 – 596.

George S, Remashree AB, Sebastian D, Hariharan M. 2004. Micropropagation of *Centella asiatica L*. through axillary bud multiplication. Phytomorphology. 54: 31-34.

Iskander MN, Song Y, Coupar IM, Jiratchariyakul W. 2002. Antiinflammatory screening of the medicinal plant *Gynura procumbens*. Plant Food Hum Nutr. 57(3-4): 223-244.

Islam MM, Haque ME, Alam SMM, Islam MA, Khalekuzzaman M, Sikdar B. 2013. Morphological and Histological Observation of Embryogenic Calli Derived from Immature Embryo of BRRI Dhan28 (*Oryza sativa L*.) Variety. Res. in Plant Biol., 3(5): 21-27.

Islam MM, Roly ZY, Lee Y and Khalekuzzaman M. 2014. *In vitro* Propagation and Genetic Transformation System Using Immature Embryo in Elite Rice (*Oryza sativa L*.) Cultivars. The Korean Society of Breeding Science. 2(1): 88-96.

Jiratchariyakul W, Jarikasem S, Siritantikorn S, Somanabandhu A, Frahm AW. 2000. Antiherpes Simplex Viral Compounds from *Gynura procumbens*. Merr. (No.498) Mohidul University Annual Research Abstracts 28: 182.

Li WL, Ren BR, Zhuo M, Hu Y, Lu CG, Wu JL, Chen J, Shun S. 2009. The anti-hyperglycemic effect of plants in genus gynura cass. The American Journal of Chinese Medicine 37(5): 961- 966.

Mahmood AA, Mariod AA, Al-Bayaty F, Abdel-Wahab SI. 2010. Anti-ulcerogenic activity of *Gynura procumbens* leaf extract against experimentally-induced gastric lesionsin rats. Journal of Medicinal Plant Research 4(8): 685- 691.

Murashige T and Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiological Plantarum. 15(3):473-497.

Patnaik J and Debata BK. 1996. Micropropagation of *Hemidesmus indicus (L.)* R. Br. Through axillary bud culture. Plant Cell rep. 15(6): 427-430.

Puangpronpitag D, Chaichanadee S, Naowaratwattana W, Sittiwet C, Thammasarn K, Luerang A, Kaewseejan N. 2010. Evaluation of nutritional value and antioxidative properties of the medicinal plant *Gynura procumbens* extract. Asian Journal of Plant Sciences 9(3): 146- 151.

Raghu AV, Martin G, Priya V, Geetha SP, Balanchandran I. 2007. Low cost alternatives for the micropropagation of *Centella asiatica* J. Plant Sci. 2(6): 592-599.

Rosidah MFY, Sadikun A, Ahmad M, Akowuah GA, Asmawi MZ. 2009. Toxicology evaluation of standardized methanol extract of *Gynura procumbens*. Journal of Ethnopharmacology 123(2): 244- 249.

Sahoo Y, Pattnaik SK, Chand PK. 1997. *In vitro* clonal propagation of an aromatic medicinal herb *Ocimum basilicum L.* (sweet basil) by axillary shoot proliferation. In Vitro Cellular and Developmental Biology - Plant 33(4):293–296.

Usha PK, Benjamin 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 (2): 102-107.