Plant Tissue Cult. & Biotech. **27**(2): 207-216, 2017 (December) **PTC&B**

High Frequency *In vitro* Regeneration of *Gynura procumbens* (Lour.) Merr.

Tanjina Akhtar Banu, Barna Goswami, Shahina Akter, Mousona Islam, Tammana Tanjin, Ahashan Habib and Salim Khan*

Plant Tissue Culture Section, Biological Research Division, Bangladesh Council of Scientific and Industrial Research, Dhanmondi, Dhaka-1205, Bangladesh

Key words: Gynura procumbens, High frequency, Regeneration

Abstract

An efficient rapid *in vitro* regeneration protocol was described from nodal segment, leaf and petiole explants. MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l IAA was found best for the multiple shoot formation from nodal segments. In this combination 99% explants produced multiple shoots and the average number of shoots per explants was 20.1 ± 1.96. For petiole and leaf explants best response was observed on MS supplemented with 2.0 mg/l BAP, 1 mg/l IAA and 0.5 mg/l Kn. Petiole explants produced highest mean number of shoots/explant (22.9 ± 1.72**8**) among the three explants when the explants were cultured on MS with 2.0 mg/l BAP, 1 mg/l IAA and 0.5 mg/l Kn. The highest frequency of root induction (100%) and mean number of roots/plantlets (11.75) were obtained on MS. The rooted plantlets were transferred for hardening following acclimatization and finally were successfully established in the field.

Introduction

Gynura procumbens is a medicinal plant belongs to Asteraceae. It is commonly found in Asian countries such as China, Thailand, Indonesia, Malaysia and Vietnam (Alizah and Nurulaishah 2014). Traditionally, it is widely used in many countries for the treatment of a wide range of health ailments such as kidney discomfort, rheumatism, *Diabetes mellitus*, constipation, and hypertension (Keng et al. 2009).

Some phenolic compounds are isolated and identified from this plant. Valuable proteins in the leaves were osmotin like protein I and thaumatin like protein (Hew and Gam 2010). The commonly occurring phytosterol in *G. procumbens* are stigmasterol, sitosterol and campesterol, both as free sterols and

*Author for correspondence: <k2salim@yahoo.com>.

as simple glycosides. Phytosterol are recognized as a hypocholesterolemic agent and are used in the treatment of atherosclerosis due to their poor absorption and competition with cholesterol for absorption sites in the intestine. *G. procumbens* extract exhibit chemo suppression effect toward malaria parasite, virucidal activity and antibacterial activity. Extracts of this plant had an enhancing effect on glucose uptake in 3T3 adipocyte cell lines and also had an anti-diabetic action through the stimulation of glucose uptake reported by Boheri et al. (2006). Leaf extracts of *G. procumbens* significantly suppress the elevated serum glucose level and reduce the serum cholesterol and triglyceride level in diabetic rats discovered by Zhang and Tan (2000). Consequently this plant has great medicinal significance.

Gynura procumbens has been long used as ethno-herbal products to treat various ailments, now in the food industry it has been incorporated into products such as tea, kimchi, coffee powder, chocolate, candy and chewing gum. The applications of *G. procumbens* in personal care and cosmetic products have also been reported which including hand-washing solution, hand sanitizer, oral spray, facial masks, and skin care creams. These products have demonstrated the high commercial value of *G. procumbens* and its variety of uses in a number of industries. (http://www.ashitabaplant.com).

The conventional propagation method of this plant is cuttings which cannot give sustainable supply of raw materials to produce various types of pharmaceutical, dermaceutical, food and aroma-therapeutcal products. That is why, in order to maintain a sufficient supply of raw material in manufacturing of *G. procumbens* products, propagation of this plant on a large scale or commercial exploitation plant tissue culture technique is greatly needed.

The present study was undertaken to establish areproducible *in vitro* regeneration protocol using different type of explants (nodal, leaf and petiole) for large-scale production of *G. procumbens* followed by conserving biodiversity and commercial propagation of medicinally important plants *Gynura* in outdoor field condition. Some reports are available on *in vitro* studies of *G. procumbens* plant using node and shoot tip explants. But to our knowledge there was no report available on *in vitro* plant regeneration of *G. procumbens* from segments of young leaf and petiole.

Materials and Methods

Three types of explants (Node, young leaf and petiole segments) were used for the present experiment. Explants were collected from field grown plants of BCSIR research field. Surface sterilization procedure of explants was followed according to the protocol described by Khan et al. (2016). Sterilized explants were

inoculated and cultured on MS containing BAP, NAA, IAA, Kn, 2,4-D and IBA singly or in combinations for direct and indirect (through callus) in *vitro* regeneration of shoots. *In vitro* regenerated shoots were sub-cultured regularly to fresh medium at an interval of 21 - 28 days for further multiplication. Elongated shoots were separated and cultured on rooting medium for root formation. About 1 - 3 cm long shoots were separated and cultured on rooting medium containing MS and half strengths of MS alone or with combination of IBA. All *in vitro* grown cultures were maintained under illumination on a 16 hrs photoperiod at 25 ± 2ºC. The well rooted plantlets were then kept in room temperature for 2 - 3 days and transferred to plastic pot containing garden soil and compost in ratio of 2 : 1 and moist them adequately for proper hardening.

Results and Discussion

A simple, effective and reproducible protocol was developed for *in vitro* regeneration of *Gynura procumbens.* Three types of explants, namely node, leaf and petiole segments were cultured on MS supplemented with various concentrations of BAP, IAA, 2, 4-D, NAA, Kn and IBA singly or in combinations to evaluate their effect on direct and indirect shoot regeneration. Explants (node, leaf and petiole segment) of *G. procumbens* showed differential response towards shoot regeneration (Tables 1 and 2).

Among the explants nodal segment showed highest response (99%) towards shoot initiation. The results of the experiments using nodal segments cultured on MS media with different hormonal combinations are presented in Table 1. Using nodal explants the best response was observed on MS supplemented with 1.0 mg/l BAP and 0.5 mg/l IAA (Table 1). Initiation of shoots was found to occur within 4 - 8 days of inoculation (Fig. 1a). The formation of multiple shoots is presented in (Fig. 1b). The regenerated shoots were found to elongate in same media combination within 45 - 60 days (Fig 2a). The mean number of shoots/explants was 20.1 ± 1.96. In this combination (1.0 mg/l BAP and 0.5 mg/l IAA) multiple shoots were also found to initiate from the inter-node portion (base of explants) (Fig. 1c). Multiple shoots which were formed from the inter-nodal portion of explants were sub-cultured in same medium combination and huge multiple shoots produced within 60 - 75 days (Fig. 1d). In case of *Piper nigrum,* Khan (2017) demonstrated that best shoot proliferation response was observed from nodal explants in MS with a combination of 1.0 mg/l BAP and 1.0 mg/l IAA. Rani et al. (2014) also got the best results on MS supplemented with 3.0 mg/l IAA and 3.0 mg/ l BAP in case of *Rauvolfia serpentina* using same explants.

Table 1. Effect of MS supplemented with different concentrations of hormones on shoot regeneration from nodal explants of *Gynura procumbens*.

Hormonal supplements(mg/l)					% of explants showed shoot regeneration	Av. no. of shoot buds/explants after 60 days	Days for shoot initiation
BAP	NAA	IAA	Kn	2,4-D			
1.0	-	-		-	60.00	6.3 ± 1.63	5 - 8
2.0	-	-		-	75.00	12.0 ± 2.94	4 - 10
3.0	**-**	**-**		**-**	**90.00**	**15.3 ± 3.09**	**6 - 10**
2	-	1	0.5	-	70	9.2 ± 2.09	8 - 10
1.0	0.2	-	-	-	85	6.3 ± 1.16	6 - 13
1.0	0.5	-	-	-	75	9.2 ± 1.47	7 - 10
1	2	-	-	-	45	5.5 ± 1.17	5 - 9
1.0	-	-		0.5	50	5.5 ± 1.17	7 - 10
1.0	-	-		0.2	60	4.9 ± 0.99	5 - 8
1.0	-	-		0.1	65	5.4 ± 1.42	6 - 10
1.0	**-**	**0.5**		**-**	**99**	**20.1** ± 1.96	**4 - 8**
1.0	-	0.2		-	70	10.7 ± 2.49	4 - 7

This study also showed that BAP is very efficient in induction of multiple shoots from nodal segment; MS containing 3.0 mg/l BAP (Table 1) was found to be the most effective for induction of multiple shoots in *Gynura procumbens.* The percentage of responsive explants was 90 towards shoot induction and mean number of shoot was 15.3 ± 3.09 on these responsive nodal explants. Shoots were formed in a cluster, when sub-cultured in low BAP (0.5 mg/l) supplemented MS then the shoots were proliferated properly (Fig. 1e, f). Keng et al. (2009) indicated that BA played an important role in induction of multiple shoot formation and was very effective in shoot proliferation and the initiation of auxillary branching of nodal explants of *Gynura procumbens* occurred on MS supplemented with 2 mg/l BA. Parvin et al. (2014) reported the best result (90%) on MS with 1 mg/l BAP from shoot tip explants. Alizah and Nurulaishah (2014) reported an average number of 3 - 8 shoots/explant from each nodal segment.

In a separate set of experiments, leaf and petiole explants were cultured with MS supplemented with different auxins and cytokinin singly or in combination for shoot induction (direct and indirect through callus formation). The results of the best response of the explants are presented in Table 2. When combinations of BAP (1.0 and 3.0 mg/l), 2, 4-D (1, 2, 3 and 4 mg/l) were applied with basal MS large callus (friable white colored with purple root formation as well as compact black coloured callus without root) was observed from both explants (leaf and petiole segments). Friable callus with purple root formation are presented in Fig. 1g. When experiments were carried out using BAP and 2,4-D initiation of shoot was observed from leaf explants via callus induction along with

Table 2. Response of leaf and petiole explants towards shoot regeneration on MS with hormonal combinations.

Hormonal supplements (mg/l)						% of respon-sive explants	Type of regener ation*	Days required to get response	Av. no. of shoot buds/explants after 60 days	
BAP	IAA	NAA	Kn	IBA	2,4-D				Leaf explants	Petiole explants
-	-	-	-	-	1	66.66	FCPR	14 - 16	-	-
-	-	-	-	-	2	80	CBC	14 - 18	-	-
1	-	-	-	-	3	60	FWC	14 - 20	3.9 ± 1.10	-
1	-	-	-	-	4	45	CBC	15 - 18	N/S	N/S
1	-	-	-	-	-	N/R	-	-	-	-
1	-	2	-	-	-	20	Shoot	15 - 20	3.4 ± 0.9 6	4.1
1	0.5	-	-	-	-	N/R	-	-	-	-
1	-	-	1	-	-	33.3	Shoot	18 - 20	2.6 ± 0.69	5
2	-	-	-	-	-	N/R	-	-	-	-
1	1	0.2	-	-	-	N/R	-	-	-	-
2	0.5	-	-	-	-	N/R	-	-	-	-
2	-	-		1	-	N/R	-	-	-	-
2	-	0.2	-	-	-	N/R	-	-	-	-
2	**1**	**-**	**0.5**	**-**	**-**	**95**	**Shoot**	**14 - 18**	**16.2** ± 1.81	**22.88**
2	-	-	1	-	-	N/R	-	-	-	-
-	1	-	1	-	-	N/R	-	-	-	-
1	1	1	-	-	-	N/R	-.	-	-	-
1	-	-	1	-	2	N/R	-	-	-	-
3	1	-	-	1	-	60	FC	15-18	N/S	3.1
3	-	-	-	1	-	N/R	-	-	-	-

*FCPR (Friable callus with purple root formation), CBC (Compact black coloured callus with root formation), FWC(Friable white callus without root formation), FC(Friable callus formation).

the formation of purple roots in MS supplemented with 1.0 mg/l BAP and 3.0 mg/l 2, 4-D (Table 2, Fig. 1h). In this combination of BAP and 2, 4-D, initiation of shoots was found to occur within 14 - 20 days of inoculation. The percentage of responsive explants was 60 and average number of shoots/explants was 3.9 ± 1.10 (Table 2). In the present study, it was noticed that leaf and petiole explants mostly showed profuse callus initiation instead shoot induction. Fastest callogenic response was also observed by Sen et al. (2014) when 2, 4-D (2.0 mg/l) and BAP (0.5 mg/l) were added to MS medium for *Achyranthes aspera*. To get better response towards shoot initiation both the explants (leaf and petiole) were further tried with other auxins and cytokynin combinations with MS basal medium.

It was observed that, nodal segment showed highest response towards shoot initiation, but maximum number of direct multiple shoot formation was observed from petiole segment on MS supplemented with 2.0 mg/l BAP, 1 mg/l IAA and 0.5 mg/l Kn. In this combination of BAP, Kn and IAA, 95% explants

showed shoot initiation (Fig. 1i) and mean number of shoot/explant was 22.9 ± 1.728(Fig. 2a, Table 2) after 60 days of culture. The highest frequency of shoot regeneration (74.75%) from petiole was obtained on MS supplemented with 0.50 mg/l BAP and 0.20 mg/l IAA in plantlet regeneration of *Populus deltoids* reported

Fig. 1. Different stages of *in vitro* shoot regeneration of *Gynura procumbens* using different type of explants : a. Initiation of shoot from nodal explants on MS supplemented with 1.0 mg/l BAP and 0.5 mg/l IAA. b. Multiple shoot formation from same explants and media combinations mentioned as Fig a. c. Initiation of multiple from the inter nodal portion of nodal explant (arrow) on MS + 1.0 mg/l BAP and 0.5 IAA. d. Proliferation of multiple shoots after 60 days on same medium mentioned as Fig. a. e & f. Multiplication and elongation of shoots from nodal explants on MS + 3 mg/l BAP. g. Friable callus formation with purple roots (arrow) from leaf explants on MS + 1 mg/l 2,4-D. h. Induction of shoots via callus formation (along with purple roots) from leaf explants on MS + 1 mg/l BAP + 3 mg/l 2,4-D. i . Initiation of shoots from petiole explants on MS + 2 mg/l BAP + 0.5 mg/l Kn + 1 mg/l IAA. j. Initiation of shoots from leaf explants on same medium mentioned as in Fig i. k & l. Multiplication of shoots from leaf and petiole explants, respectively on MS with 2 mg/l BAP, 0.5 mg/l Kn and 1 mg/l IAA.

by Thakur et al. 2012. Nodal segments also showed 70% (Table 1) response on the same medium combination (2.0 mg/l BAP, 1.0 mg/l IAA and 0.5 mg/l Kn) whereas the mean number of shoot was 9.2 ± 2.09 after 60 days of culture. An optimum number of 5 - 10 shoots per nodal explant were also obtained in 6 weeks using 3.0 mg/l BAP, 0.5 mg/l 2-ip and 1.0 mg/l IAA in *Adhatoda beddomei* (Sudha et al. 1994). Leaf explants also showed good response on the same media combination (2.0 mg/l BAP, 1 mg/l IAA and 0.5 mg/l Kn) where the mean number of shoots per explant was 16.2 ± 1.8 (Fig. 2b). Shoot initiation found to occur after 18 days of inoculation in case of leaf explants (Fig. 1j) whereas, initiation of shoots found to occur after 14 days of inoculation incase of petiole explants. Mollika et al. (2011) also reported best result in *Brassica* sp. using cotyledonary leaf explants in MS supplemented with 2 mg/l BAP, 0.2 mg/l NAA and 0.5 mg/l Kn. Direct shoot initiation (Fig. 1g, h) and multiple shoot formation from petiole and leaf explants in this media combination are presented in Fig. 1k, l. The regenerated shoots were routinely sub-cultured for further multiplication. Multiple shoots were largely elongated and proliferated after 60 days.

Table 3. Effect of half and full strengths of MS and with IBA for root induction from the *in vitro* regenerated shoots.

Supplements	% of responsive shoots for root induction	Days to root induction	Mean No. of roots/plantlets
Half strength of MS	100	5-10	12
Full strength of MS	100	4-9	11.75
Full strength of MS + 0.1 mg/l IBA	100	6-13	15.7

Regenerated shoots (2- 3 cm) were isolated and used for root induction. For this reason MS and half strength of MS media alone and sometimes with various concentrations and combinations of IBA were used for root induction from *in vitro* grown shoots. Among the all combinations MS showed most early rooting response (100%), where 4 - 9 days were required for root initiation and mean number of roots per plantlet were 11.75 (Fig. 2c, d). However, higher mean number of roots per plantlet (15.7) was obtained on MS supplemented with 0.1 mg/l IBA (Table 3). Alizah and Nurulaishah (2014) reported similar observation. They observed that MS without the addition of any auxin was sufficient for the establishment of *in vitro* rooting of *G. procumbens*. Morimoto et al. (1994), Cuenca and Amo-Marco (2000), Akter et al. (2013), obtained the similar result in case of *Salvia miltiorrhiza*, *Salvia bancoana* and *Salvia valentine,* respectively and in *Aegle*

marmelos. The maximum rooting response (100%) of *Gynura procumbens* was achieved on MS supplemented with NAA (0.5 mg/l) according to Parvin et al. (2014).

After sufficient development of roots, rooted plantlets were transferred to plastic pots filled with soil and kept in culture room (Fig. 2e). In the present study, 100% transplanted plantlets survived in the field. For their further growth and establishment the plantlets were transferred to small field plots. Hence, it can be used for the large scale production of this medicinal plant within a short time.

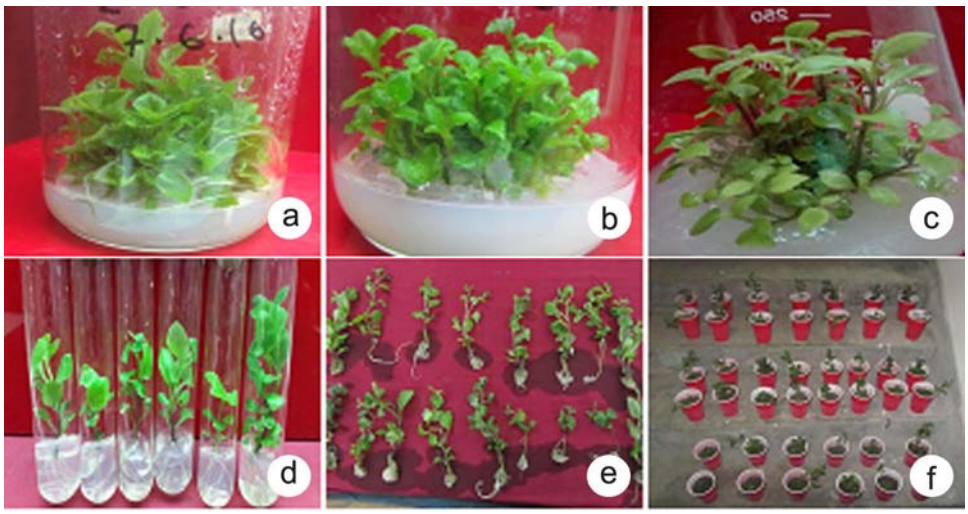

Fig. 2. *In vitro* regenerated shoots and formation of roots from *in vitro* regenerated shoots of *Gynura procumbens.* a. Elongation of regenerated shoots from nodal segment on MS with 1.0 mg/l BAP and 0.5 mg/l IAA; b. Shoot elongation from petiole explants on MS + 2 mg/l BAP + 0.5 mg/l Kn + 1 mg/l IAA. c. Mature shoots from leaf explants on same medium mentioned as in Fig b. d. Formation of roots from *in vitro* regenerated shoots on full strength of MS. e. *In vitro* regenerated plantlets were prepared for hardening. f. Hardening of *in vitro* regenerated plantlets in small plastic pot.

It can be concluded that, the micropropagation protocol reported in the present study was characterized with a rapid proliferation of shoots using various explants (node, leaf and petiole) , easy rooting of the micro-shoots and the plantlets were easily acclimatized to the external environment and undergoing normal physiological development. Moreover, there was no report available for the micropropagation of *Gynura procumbens* using leaf and petiole segments. So, this regeneration protocol is highly advantageous for conservation, large scale production as well as uniform source of *Gynura procumbens* plants to

yield important secondary metabolites for pharmaceutical industries with a range of further biotechnological applications.

References

Akter S, Banu TA, Habib A Afrin, S Khatun, A Khan S and **Islam S** (2013) *In vitro* clonal multiplication of *Aegle marmelos* (L.) Corr. through cotyledonary node culture. Banladesh J. Sci. Ind. Res. **48**(1): 13-18.

Alizah Z and **Nurulaishah Y** (2015) Multiple shoot regeneration from nodal explants of *Gynura procumbens* (Lour.) Merr Z. Annual Research & Review in Biology **6**(2): 85-88.

Bohari M, Pauliena S, Muhajir H, Khozirah S and **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.

Cuenca S and **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.

Hew CS and **Gam IH** (2010) The identification of high abundant proteins in the leaves of gynura procumbens, Biotechnol & Biotechnol. V10133-010-0072-9.

Keng CL, Yee LS and **Pin PL** (2009) Micropropagation of *Gynura procumbens (*Lour.) Merr. Animportant medicinal plant. J. Medicinal Plants Res. **3**(3): 105-111.

Khan S, Akter S, Habib A, Banu T A, Islam M, Khan N F, Afrin S, Ferdousi A and **Islam S** (2016) Establishment of *in vitro* regeneration protocol for *Adhatoda vasica* Nees. Bang. J. Sci. Ind. Res. **51**(1): 75-80.

Khan S, Banu TA, Habib A, Islam M, Ferdousi A, Das N and **Akter S** (2017) *In vitro* regeneration of *Piper nigrum*. Bangladesh J. Bot. **46**(2)**:** 789-793.

Mollika SR, Sarker RH and **Hoque MI** (2011) *In vitro* Plant Regeneration in *Brassica* spp. Plant Tissue Cult. & Biotech. **21**(2): 127-134.

Morimoto S, Goto Y and **Shoyama Y** (1994) Production of litho spermic acid B and rosmarinic acid in callus tissue and regenerated plantlets of *Salvia miltiorrhiza*. J. Nat Prod. **57**: 817-823.

Parvin F, Islam J, Jahan N and **Rahman M** (2014) Efficient *in vitro* micropropagation of *Gynura procumbens*- an important rare medicinal plant, through shoot tip and nodal segment explants. Journal of Research in Biology, Online: 2231- 6299.

Rani A, Kumar M and **Kumar S** (2014) Effect of growth regulators on micropropagation of *Rauvolfia serpentine* (L.) Benth. J. Applied and Natural Sci. **6**(2): 507-511.

Sen MK, Nasrin S, Rahman S and **Jamal AHM** (2014) *In vitro* callus induction and plantlet regeneration of *Achyranthes aspera* L., a high value medicinal plant. Asian Pac.9 J. Trop. Biomed. **4**(1): 40-46.

Sudha CG and **Seeni S** (1994) *In vitro* multiplication and field establishment of *Adhatoda beddomei* C. B. Clarke, a rare medicinal plant. Plant Cell Rep. **13**(3-4): 203-207.

Thakur AK, Saraswat A and **Srivastava DK** (2012) *In vitro* plant regeneration through direct organogenesis in *Populus deltoids* clone G48 from petiole explants. J. Plant Biochem. Biotechnol. **21**(1): 23-29.

Zhang XF and **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.

http://www.ashitabaplant.com