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In Vitro Regeneration and *Ex Vitro* Establishment of an Antidiabetic Plant- *Gynura procumbens* (Lour.) Merr.

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Abstract: *A most effective protocol for axillary shoot proliferation was established on MS medium supplemented with different concentrations of cytokinin and auxin using nodal and shoot tip explants from field grown mature plant of Gynura porcambens. In vitro response of the explants to multiple shoot regeneration varied greatly with the position of the explanting branch on the donor plant. Highest frequency 98.21% of shoot formation, maximum number 6.2 of shoots per explant and average 4.5 cm length of shoot were obtained on MS medium supplemented with 4.0 µM 6-Benzylaminopurine (BAP). Shoot multiplication and growth were significantly affected by level of concentration of sucrose. Optimum sucrose concentration was also estimated for in vitro shoot regeneration from nodal explant in MS medium with different concentrations of sucrose. The highest rate (97.31%) of shoot production was achieved in a shoot-regenerating medium with 30 gm/l sucrose. For rooting, the in vitro proliferated and elongated shoots were excised into 2–4 cm long microcuttings, which were planted individually on a root-induction MS medium containing 4.0 µM Indole-3-butyric acid (IBA). Within 4 weeks of transfer to the rooting medium, all the cultured microcuttings produced 2–6 roots. The in vitro regenerated plantlets were transferred to soil, and the survival rate ex vitro was 95.00%.*

Keywords: *Medicinal Plant, Nodal Segments, Plant Growth Regulators, Sucrose.*

1. INTRODUCTION

Gynura procumbens Merr. belonging to the family Asteraceae is a medicinal plant commonly found in tropical Asia countries such as China, Thailand, Indonesia, Malaysia, and Vietnam. Traditionally, it is widely used in many different countries for the treatment of a wide variety of health ailments such as kidney discomfort, rheumatism, diabetes mellitus, constipation, and hypertension. Based on the traditional uses of *G. procumbens*, it seems to possess high therapeutic potential for treatment of various diseases making it a target for pharmacological studies aiming to validate and provide scientific evidence for the traditional claims of its efficacy. Recently, pharmacologic studies reported that *G. procumbens* has anti–Herpes simplex virus, anti–hyperglycemic, anti–hyperlipidemic, anti–inflammatory, analgesic, and reduced blood hypertension properties (Lam *et al.*, 1998; Akowuah*et al.*, 2001, 2002; Iskander*et al.*, 2004).

This plant is conventionally propagated by stem cuttings. The conventional method cannot meet the increasing demandof 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 *Centellaasiatica* (Tiwari *et al.*, 2000), *Vitisthunbergii* (Mei, 2005) and even woody medicinal plant, *Garcinia indica* (Malik *et al.*, 2005), *Phellodendronamurense* (Azad *et al.*,2012) also.

By considering the medicinal value and antidiabetic properties of *G. procumbens* the present project has been undertaken for develop a suitable micropropagation protocol for the mass production of this plant species. Here, we will try to establish 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 will be followed by the optimized conditions for *in vitro* rooting and further transfer into the greenhouse. To our knowledge, there are few reports on micropropagation of this plant species.

2. Materials and Methods

Nodal and shoot tip explants of *Gynura procambens* were collected from a medicinal plant garden in Rajshahi University campus, Bangladesh. Explants were washed thoroughly under running tap water for 15 minutes and then washed with continuous agitation in a few drops Savlon containing water for 15 minutes. The washed explants were then treated with 0.1% $HgCl_2$ for 10minutes under laminar air flow cabinet to disinfect them. Finally, explants were washed 3 to 5 times with sterile distilled water and were placed in culture tubes (25 × 150 mm) containing 4.0 µM BAP supplemented MS (Murashige and Skoog, 1962) medium prepared with 3% (w/v) sucrose and 0.8% (w/v) agar (Sigma Chemical Co. USA). The pH of the medium was adjusted to 5.7 ± 1 before autoclaving at 121°C for 20 minutes at 1.2 kg/cm^2 pressure.

To test the effect of basal medium on *in vitro* shoot multiplication, nodal explants were initially cultured on four different basal medium viz. MS (Full strength of MS medium), MMS1 (Half strength of MS medium), MMS2 (Quarter strength of MS medium) and B5 (Gamborged*et al*., 1968) supplemented with 4.0 µM BAP. In addition, excised nodes of *in vitro* grown shoots were cultured on MS medium containing various concentrations (2.0-6.0 µM) of BAPalone or in combination of 1.0 µM of IBA (Indole-3-butyric acid) orNAA (1-Napthaleneacetic acid) to test their shoot induction efficiency. Nodal explants were also used for examining the effects of the sucrose concentrations on shoot regeneration. Four different concentrations (10.0, 20.0, 30.0 and 40.0 gm/l) of sucrose in a medium were employed for the experiments. Microshoots of 1-3 cm length were prepared from usable shoots by snipping off the basal leaves and cultured them individually in 25 × 150 mm culture tubes with 10-15 ml of full strength MS medium supplemented with2.0-6.0 µM of IBA,NAA, or IAA (Indole-3-acetic Acid)

The rooted plantlets were transferred on to the small plastic pots containing sterilized soil mix (garden soil: sand: compost in 2:1:1 ratio). Transferred plantlets were hardened in growth chamber condition for 20 days and then transferred to outdoor condition. The total number of plants transferred to the pots and the number of surviving plants in the outdoor condition were recorded. All the cultures were maintained at 25 ± 1°C under a 16h light and 8h dark cycle with the light intensity of 2000-3000 lux provided by cool-white fluorescent tubes (36 W). Data were recorded after 8 weeks of culture except for rooting experiment when the data were recorded after 4 weeks of incubation. In all the experiments, 12-15 explants were used and each experiment was repeated three times. Mean and standard error were calculated for all numerical data. The mean data of each treatment were compared by using Duncan's Multiple Range Test (DMRT) at P=0.05%.

3. Results and Discussion

Nodal explants showed the best shoot proliferation efficiency irrespective of media type, which followed by shoot tip explants (Fig. 1). Nodal explants showed the maximum 98.21 ± 1.01% response and produced 6.22±0.51 shoots and average length of shoots 4.51±0.24 cm on MS medium with 4.0 µM BAP while the shoot tips formed maximum 3.11 ± 0.62 shoots, and 3.24 ± 0.71 cm shoot length,

respectively on the same media formulation (Fig. 2A-B). Results of this experiment indicated the high regenerative capacity of nodalexplants of *G. procambens* than shoot tip explants. On the other hand Parvin*et al*. (2014) reported that shoot tip explant was superior to nodal explant for multiple shoot induction. Our findings were similar to the medicinal species, such as *G. porcambens* (Alizah and Nurulaishah, 2014, Keng*et al*., 2009; Majumder*et al*., 2016); *Phaleriamacrocarpa* (Abdullah *et al*., 2014);*Medinillamandrakensis* (Elinorovololona and Martial, 2014), *Phellodendronamurense* (Azad *et al*., 2005); *Adhatodavaisca* (Azad *et al*., 2003) and *Ocimumbacilicum* (Begum *et al*., 2002).

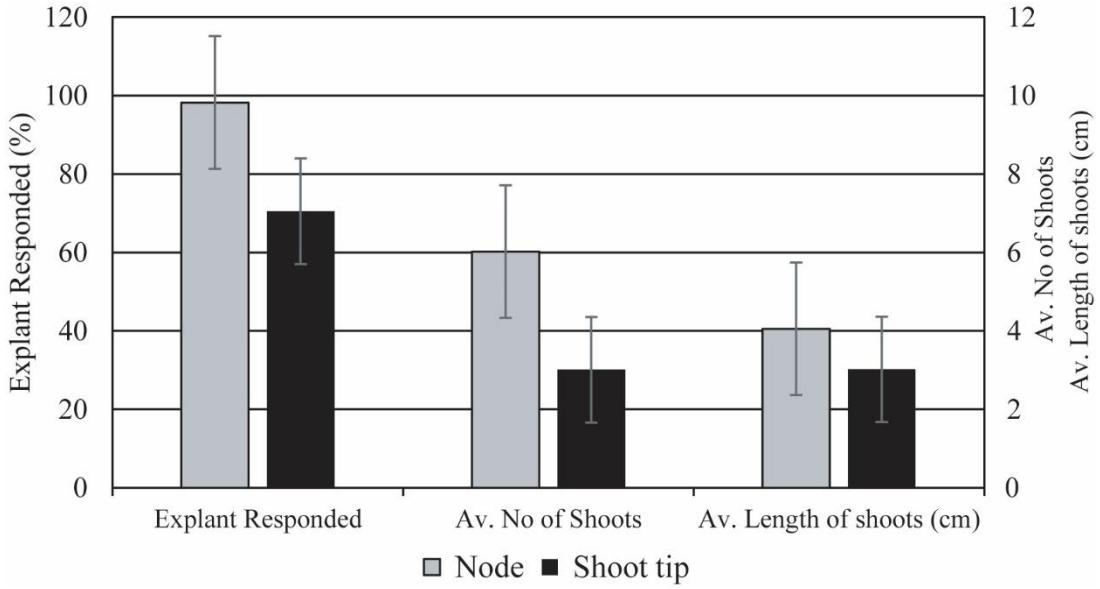

Figure 1. *Effects of explants on MS medium containing 4.0 µM BAP on axillary shoot proliferation of Gynura porcambens.*

The nodal explants significantly affectedshoot proliferation on full strength MS medium supplement with 4.0 µM BAP than other three media (MMS1, MMS2 and B5) tested (Fig. 3). Maximum 98.52 ± 1.51 % explants produced highest 6.53 ± 0.61 shoots with highest average length 4.33 ± 0.41 cm on MS medium. The second highest frequency 95.05% of shoot proliferation 6.11 ± 0.31 average number of shoot and 3.81 ± 0.42 cm length of shoot were obtained on MMS1 medium. Considerable shoot proliferation were observed on B5 medium. The lowest performance of shoot proliferation, number of shoot, and shoot length were found on MMS2 medium. This study revealed that full strength MS medium was preferred for axillary shoot proliferation from nodal explants of*G. procambens* while B5 medium showed a little effect in terms of shoot proliferation. Full strength MS medium has been proved best for axillary shoot proliferation in many other medicinal species, such as *Phaleriamacrocarpa* (Abdullah *et al*., 2014); *G. porcambens* (Alizah and Nurulaishah, 2015, Keng*et al*., 2009; Majumder*et al*., 2016); *Adhatodavasica* (Azad *et al*., 2003); *Ocimum sanctum* (Begum *et al*., 2000); and*Rauvolfiaserpentia* (Shahrear*et al*., 2002).Similar results were also observed in some other woody trees, like *Phellodendronamurense* (Azad *et al*., 2004), *Accia catechu* (Kaur *et al*., 1998), *Accianilotica* (Abbas *et al*., 2010), *Coluteaistria* (Hegazi and Gabr, 2010), *Pterocarpus marsupium* (Hussain *et al*., 2008), *P. santalinus* (Rajeswari and Paliwal, 2008), *Sterculiaurens* (Hussain *et al*., 2007) and*Lagerstromiaparviflora* (Tiwari *et al*., 2002).

Results influenced on the various concentrations of BAP alone or in combination with IBA or NAA in axillary shoot proliferation from nodal explants are given in Table 1. Nodal explants also cultured on MS medium devoid of growth regulator produced 1.2 ± 0.1 shoots, 1.4 ± 0.2 cm lengths with 50.12% shoot regeneration frequency, which may be due to the presence of endogenous cytokinin in nodal

explant, suggested by Rajeswari and Paliwal (2008) in *Pterocarpussantalinus*. However, the addition of exogenous cytokinin to MS medium induced shoot multiplication rate remarkably, indicating the requirement of exogenous cytokinin supply in the medium for better axillary shoot proliferation. Among the various concentrations (2.0-6.0 µM) of BAP the highest frequency 91.33% of shoot proliferation, 6.1 ± 0.4 number of shoots and 4.5 ± 0.2 cm shoot length were observed when nodal cultured on MS medium supplemented 4.0 µM BAP alone. These results indicated that BAP, a cytokinin, played an important role in induction of multiple shoot formation and was very effective in shoot proliferation. However, BAP at higher concentrations not only reduced the number of shoots formed but also resulted in stunted growth of the shoots. Similar synergistic effects were demonstrated in *G. porcambens* (Alizah and Nurulaishah, 2014; Keng*et al*., 2009) and *Phaleriamacrocarpa* (Abdullah *et al*., 2014).

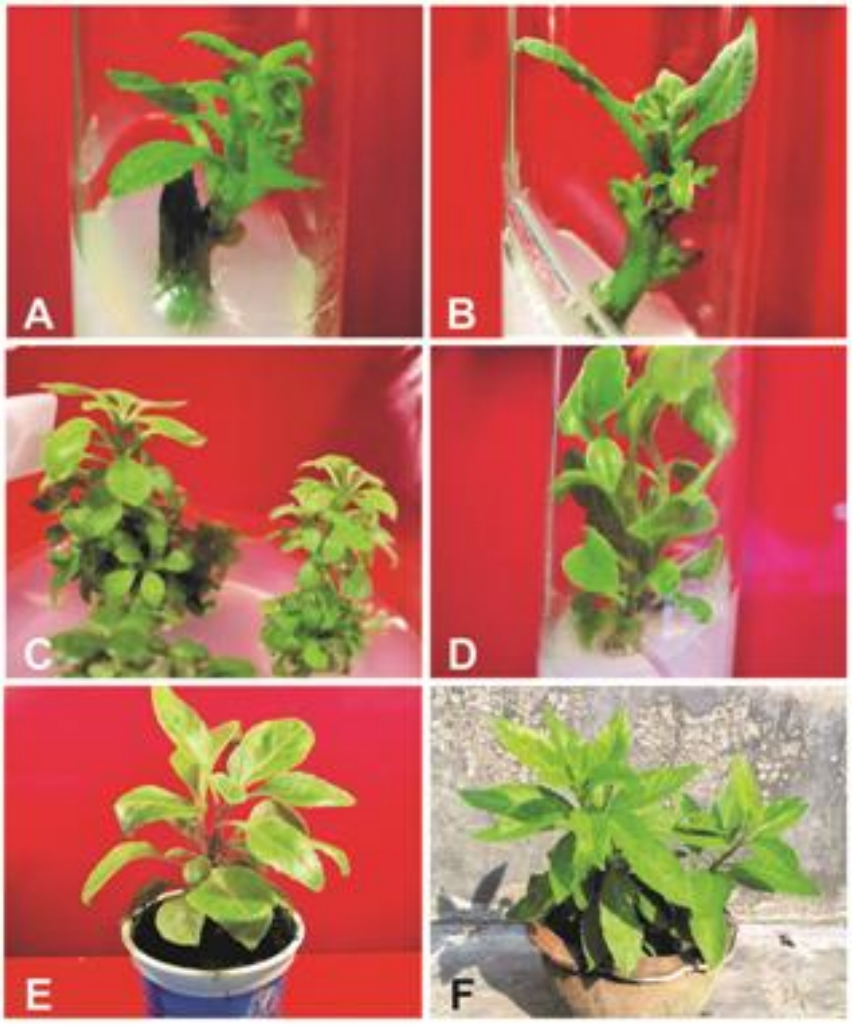

Figure 2. A-E: *In vitro* regeneration of plantlet from node and shoot tip explants of *Gynura porcambens.*

A-B: Proliferation of axillary shoots from node (A) and shoot tip (B) explants cultured on MS medium supplemented with 4.0 µM BAP after four weeks of culture.

C: Proliferation and development of multiple shoots from nodal segments on MS medium containing 4.0 µM BAP plus 1.0 µM IBA after eight weeks of culture.

D: Formation of roots from the regenerated shoots cultured on MS medium supplemented with 4.0 µM IBA.

E-F: Growth of transplant on the soil after eight weeks (E) and twelve weeks (F) of transfer under *ex vitro*conditions.

Table1. *Effects of plant growth regulators on axillary shoot proliferation from nodal explants of Gynura porcambens.*

Plant Growth Regulators (µM)	Explant responded (%)	Av. No of shoots	Av. Length of shoots (cm)
Control (HO)	50.12 ± 1.3d	1.2 ± 0.1f	1.4 ± 0.2e
BAP			
2.0	70.59 ± 1.5c	3.6 ± 0.9d	3.9 ± 0.1b
4.0	91.33 ± 1.9a	6.1 ± 0.4a	4.5 ± 0.2a
6.0	82.37 ± 1.4b	4.3 ± 0.7c	4.1 ± 0.5b
BAP + IBA			
2.0 + 1.0	72.81 ± 1.7c	3.1 ± 0.6d	3.1 ± 0.8c
4.0 + 1.0	86.39 ± 1.5b	4.9 ± 0.4b	4.2 ± 0.6ab
6.0 + 1.0	77.87 ± 1.3c	3.5 ± 0.9d	3.5 ± 0.4a
BAP + NAA			
2.0 + 1.0	69.52 ± 1.6c	2.1 ± 0.7e	2.5 ± 0.6d
4.0 + 1.0	83.67 ± 1.4b	4.1 ± 0.6c	3.8 ± 0.3b
6.0 + 1.0	75.33 ± 1.7c	2.5 ± 0.9e	2.9 ± 0.4c

Values represent means ± standard error of 12-15 explants per treatment in four repeated experiments. Means followed by the same letter are not significantly diff erent by the Tukey's multiple comparison test at 0.05 probability level.

BAP and IBA, BAP and NAA combinations also affected normal growth and proliferation of axillary shoots. The maximum of 86.39% explants produced normal shoots with 4.9 ± 0.4 shoots and 4.2 ± 0.6 cm shoot length when they were cultured with 4.0 µM BAP and 1.0 µM IBA (Fig. 2C). Other combinations of BAP and IBA in the initial media greatly altered the growth behaviour of the cultured explants that proliferated axillary shoots. BAP-NAA combination was also produced better result for shoot proliferation. In this formulation, MS medium supplemented with 4.0 µMBAP and 1.0 µMNAA was found to be the effective medium for shoot proliferation resulting in the formation of an average of 4.1± 0.6 shoots per explant and average shoot length 3.8 ± 0.3 cm. This is in agreement with the result of *G. porcambens* (Keng*et al*., 2009), *Santolinacanescens* (Casado*et al*., 2002), and *Rotulaaquatica* (Martin, 2003). Our results showed that BAP is very effective in induction of multiple shoot from nodal segment. BAP is regarded the most effective cytokinin for shoot induction and is widely used in *in vitro* propagation of plants. The enhanced rate of multiple shoot induction in cultures supplemented with BAP may be largely ascribed due to increased rates of cell division induced by cytokinin (BAP) in the terminal and axillary meristematic zone of explant tissues. Cells in this zone divide with the faster pace and thus, produced large number of shoots (Niranjan*et al*., 2010).

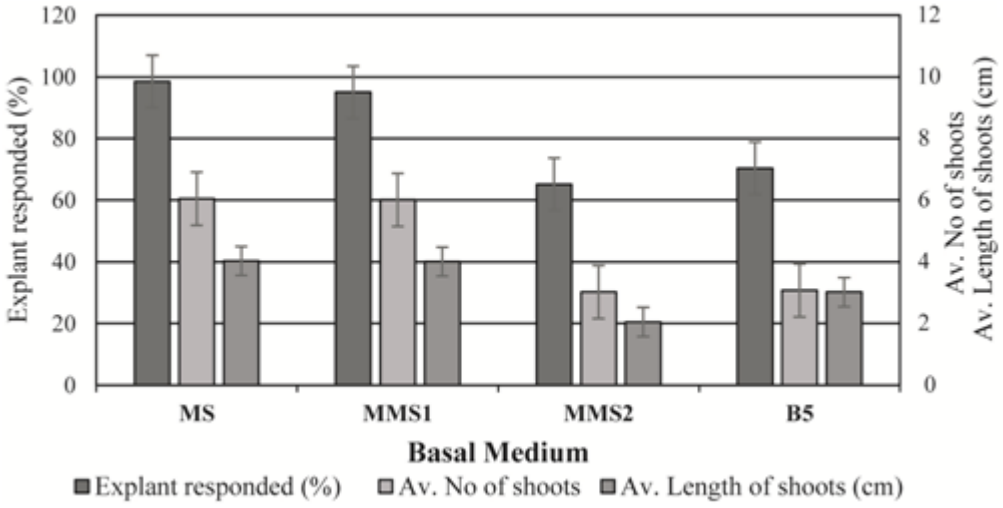

Figure 3. *Effects of different basal media containing 4.0 µM BAP on in vitro shoot multiplication from nodal explants of Gynura porcambens.*

Shoot development from nodal explant may vary among species and genotypes upon the level of optimum sucrose concentration (Nowak *et al*., 2004). The percentage of shoot formation, total number of shoots per culture and average length of shoots per culture increase gradually with the increase of the sucrose concentration in medium up to 30 gm/l. However, the performance of shoot development gradually decreased with an increase in the sucrose concentration to above 30 gm/l. The medium with 30 gm/l sucrose showed the highest percentage of shoot formation (97.3%), total number of shoots per culture (6.4 ± 0.3) and average length of shoots per culture (4.9 ± 0.4 cm) (**Fig. 4**). Anefficient carbon sources for enhanced shoot growth and development has been examined in tissue culturesof some plant species, such as *Echinacea purpurea* (Nilanthai and Yang, 2013), *Allium chinense* (Zu*et al*., 2008), *Elaeocarpusrobustus* (Rahman *et al.*, 2004) and *Paederiafoetida* (Amin *et al.*, 2003).Our study revealed that MS medium with 30 gm/l sucrose showed the best result for shoot formation in *G. porcambens*. Lower concentrations of sucrose have been shown to be less effective for shoot formation. On the other hand, the detrimental effect of a high sucrose concentration on shoot formation implies that the osmotic level in themedium may be inhibitory to further shoot development. Thus, high concentrations of sucrose seem to inhibit shoot growth and development. This observation are supported by another report (Nowak *et al*., 2004).

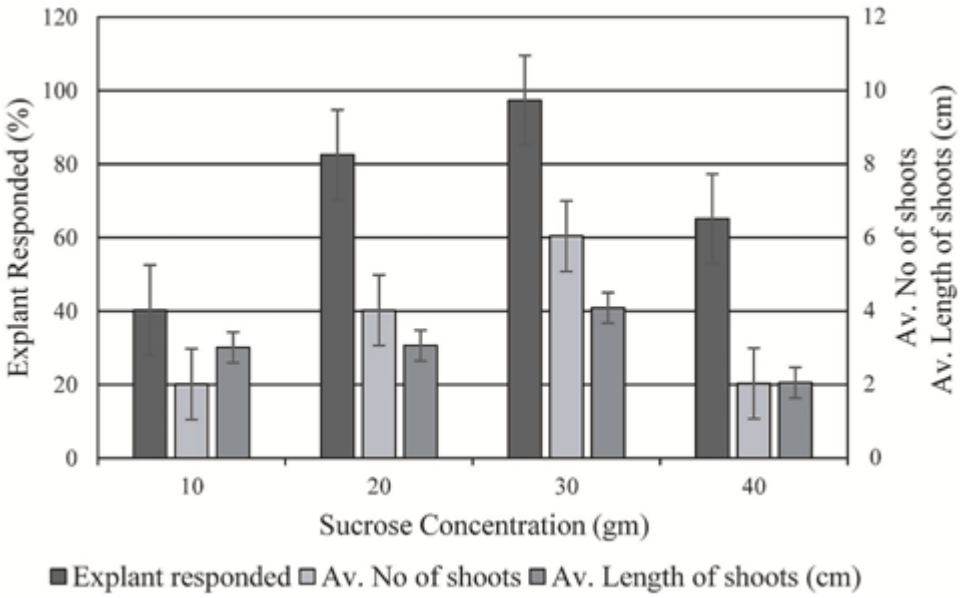

Figure 4. *Effects of sucrose concentrations on axillary shoot proliferation on MS medium containing 4.0 µM BAP of Gynura porcambens.*

Rooting frequency was enhanced considerably when either IBA, NAA or IAA at different concentrations were added to MS medium. IBA supplemented medium remarkably influenced the rate of root induction than NAA or IAA(Fig. 5). Among the different concentrations of IBA tested, highest frequency (95.0%) with maximum number 4.3 ± 0.3 of roots per shoot and longest 6.2 ± 0.1 cm roots having considerable lateral roots were obtained with 4.0 µM IBA (Fig. 2D) whereas, 4.0 µM NAA produced considerable root formation where highest frequency was 86.33, average number of roots 3.4 ± 0.1, and average length of roots 4.1 ± 0.2 cm. On the other hand IAAwas found to be less effective than IBA or NAA regarding rooting of micro-shoots where the roots were thin in nature. Poor rooting was observed when micro-shoots (2-4 cm in length) were cultured on auxin free MS medium. In this study, IBA was proved to be best auxin as comparable to NAA or IAA with regard to all rooting parameters. There are many authors reported that IBA has been found suitable for rooting in many species like *Gynura porcambens* (Majumder*et al*., 2016), *Acacia nilotica* (Dhabhai*et al*., 2010), *Bauhinia variegata* (Mathur and Mukunthakumar, 1996), *Phellodendronamurense* (Azad et.

al., 2005, 2009), and *Pterocarpus marsupium* (Husain *et al*., 2008). After successful rooting of micro-shoots, attempts were taken to establish regenerated plantlets onto soil. Plantlets had been transferred to small plastic pots containing soil mix (garden soil: compost: sand, 2:1:1) and maintained under humid *ex vitro* condition in the growth room (Fig. 2E). The *in vitro* derived plantlets acclimated better under *ex vitro* condition when they were maintained in growth room for 20 days before transferring them to outdoor condition (Fig. 2F). Finally, 95% transplanted plantlets were survived and acclimated well under *ex vitro* condition after 25 days of transplantation.

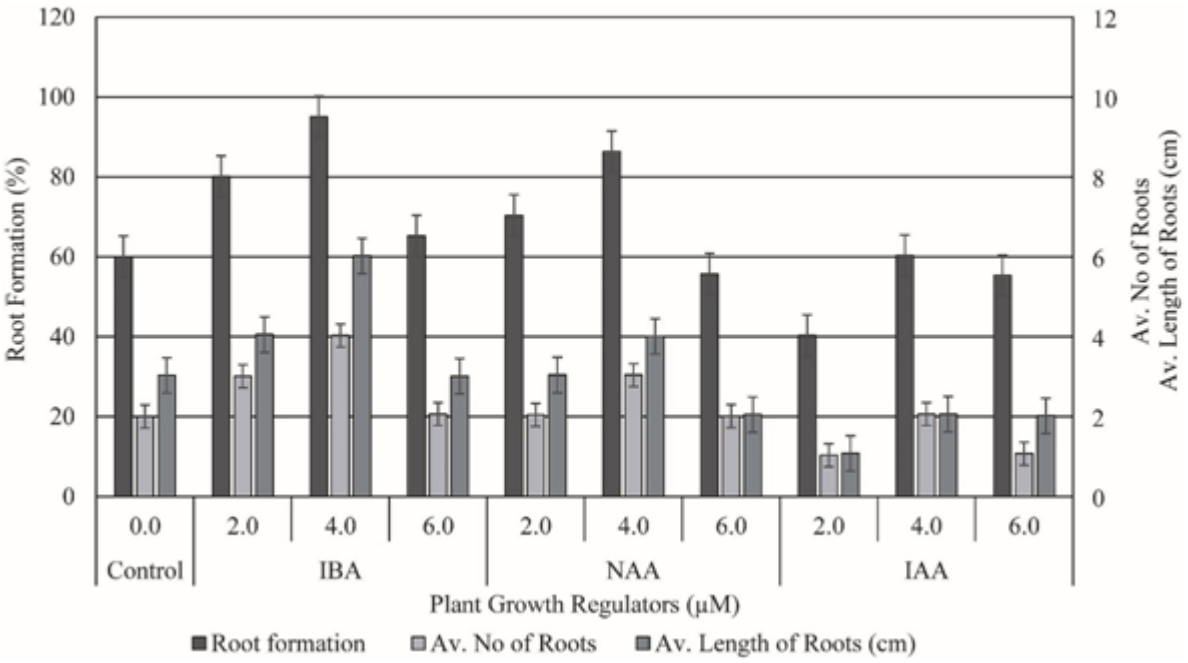

Figure 5. *Effects of auxins on adventitious rooting on MS medium of in vitro derived micro-shoots of Gynura porcambens.*

4. Conclusions

Most of the populations of world are suffering from diabetes. They are using different types of drug for controlling diabetes. These drugs are very expensive especially human insulin. Poor people are unable to buy these drugs. So they are suffering from different disease due to the diabetes. Whereas we can easily control our diabetes by eating 4-6 leaves of *G. procumbens* daily. *G. procumbens* is commonly used for diabetes treatment in traditional medicine and its hypoglycemic effect has been reported in *in vivo* studies (Hamid *et al.*, 2004; Algariri*et al.*, 2014). By considering the medicinal value and antidiabetic properties of *G. procumbens* the present project has been undertaken for develop a suitable micropropagation protocol for the mass production of this plant species. Here, we developed 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 will be followed by the optimized conditions for *in vitro* rooting and further transfer into the greenhouse. The described strategy demonstrates an efficient system of *in vitro* clonal propagation of *Gynura porcambens* via node and shoot tip explants, which could play a significant role in large scale plantlet production all around the year, as well as in wide plantation and in conservation of this plant's genetic resources.

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REFERENCES

Abbas, H., Qaiser, M.,&Naqvt, A. B. (2010). Rapid *in vitro* multiplication of *Accaianilotica*subsp. hemisperica, a critically endangered endemic taxon. *Pak. J. Bot.* 42(6):4087-4093.

Abdullah, N., Hassan, N. H., Rahman, S. S. A., Ismail, H., Khalid, R.,&Yahya, M. F. (2014): *In vitro* propagation of *Phaleriamacrocarpa,* God's Crown . *J. Biotechnol. Pharma. Res.* 5(2): 18-23.

Akowuah, G. A., Amirin, S., Mariam, A.,&Aminah, I. (2001). Blood sugar lowering activity of *Gynura procumbens* leaf extracts. *J. Trop. Med. Plant* 2: 5–10.

Algariri, K., Atangwho I. J., Meng, K. Y., Asmawi, M. Z., Sadikun, A., &Murugaiyah, V. (2014). Antihyperglycaemic and toxicological evaluations of extract and fractions of *Gynura procumbens* leaves. Trop. Life. Sci. Res.25: 75–93.

Alizah, Z.,&Nurulaishah, Y. (2015). Multiple Shoot Regeneration from Nodal Explants of *Gynura procumbens* (Lour.) Merr*Annual Res. Rev. Biol.* 6(2): 85-88.

Amin, M. N., Rahman,M. M.,&Manik,M. S.(2003). *In vitro* clonal propagation of *Paederiafoetida* L. A medicinal plant of Bangladesh. *Plant Tiss. Cult.* 13: 117-123.

Azad, M. A. K. (2012). Plant regeneration through callus-derived protoplasts of *Phellodendronamurense*Rupr. BioTechnol: *An Indian J*. 6(10): 317-326.

Azad, M. A. K., Amin,M. N.& Begum,F.(2003). Rapid clonal propagation of a medicinal plant - *Adhatodavasica*Nees. using tissue culture technique. *OnLine J. Biol. Sci*.3 (2): 172-182.

Azad, M. A. K., Yokota, S.,Yahara,S., &Yoshizawa,N.(2004). Effects of explant type and growth regulators on organogenesis in a medicinal tree, *Phellodendronamurense*Rupr. *Asian J. Plant Sci*. 3(4): 522-528.

Azad, M. A. K., Yokota,S., Ohkubo,T.,Andoh, Y.,Yahara,S.and Yoshizawa,N.(2005). *In vitro* regeneration of the medicinal woody plant *Phellodendronamurense*Rupr. through excised leaves. *Plant Cell Tissue and Organ Cult.*80(1): 43-50.

Azad, M. A. K., Yokota, S., Begum, F., &Yoshizawa, N. (2009). Plant regeneration through somatic embryogenesis of a medicinal plant, *Phellodendronamurense*Rupr. *In vitro Cell Dev Biol Plant.* 45(4):441-449.

Azad. M. A.K., Yokota, S., Ohkubo, T., Andoh, Y., Yahara, S., &Yoshizawa. N. (2005). *In vitro* regeneration of the medicinal woody plant *Phellodendronamurense*Rupr. through excised leaves. *Plant Cell Tissue Organ Cult* 80:43-50.

Begum, F., Amin,M. N.&Azad,M. A. K.(2002) *In vitro* rapid clonal propagation of *Ocimumbasilicum* L. *Plant Tissue Cult*. 12(1): 27-35.

Casado, J. P., Navarro, M. C., Utrilla, M. P., Martinez, A.,& Jimenez, J. (2002). Micropropagation of *Santolinacanescens*Lagasca and *in vitro* volatiles production by shoot explants. *Plant Cell Tissue Organ Cult.* 69:147– 153.

Dhabhai, K., Sharma, M. M.,&Batra, A. (2010). *In vitro* clonal propagation of *Acacia nilotica* (L.)-a nitrogen fixing tree. *Researcher*. 2(3):7-11.

Elinorovololona, R. N.,& Martial, E. L. (2014). Effects of growth regulators 6-Benzylaminopurine and 2-Naphtalene Acetic Acid on the *in vitro* shoot multiplication from nodal segment of *Medinillamandrakensis* (Melastomataceae). *Intl. J. Biochem. Biotechnol*. 3 (1): 504-510.

Gamborg, O. L., Miller, R. A., &Ojima, K., (1968) Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res*. 50: 151-158.

Hamid, M., Saufi, M., &Nik, M. M. (2004). Study on antidiabetic properties of *Gynura procumbens*Merr, in 18 Seminar of the Malaysian Natural Products Society (Kota Kinabalu: Universiti Malaysia Sabah).

Hegazi, G. A. E.,&Gabr, M. F. (2010). Overcoming early shoot senescence of *Coluteaistria*Miller propagated *in vitro*. *J Am Sci.* 6(12):1733-1738.

Husain, M. K., Anis, M.,&Shahzad, A. (2008). *In vitro* propagation of a multipurpose leguminous tree (*Pterocarpus marsupium*Roxb.) using nodal explants. *Acta. Physiol. Plant.* 30:353-359.

Hussain, T. M., Chandrasekhar, T., &Gopal, G. R. (2007). High frequency shoot regeneration of *Sterculiaurens*Roxb. an endangered tree species through cotyledonary node cultures. *Afr. J. Biotech.* 6(14):1643-1649.

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

Kaur, K., Verma, B., &Kant, U. (1998). Plants obtained from the khair tree (*Acacia catechu*Willd.) using mature nodal explants.*Plant Cell Rep*. 17:421-429

Keng, L., Lim, S. Y., & Pan, L. P. (2009). Micropropagation of *Gynura procumbens* (Lour.) Merr. an important medicinal plant. *J. Medicinal Plants Res.* 3(3):105-111.

Lam, S. K., Idris, A., Bakar, Z. A. A., &Ismail, R. (1998). *Gynura procumbens* and blood pressure in the rat: Preliminary study. *Asia Pac J Pharmacol.* 13: S14–15.

Majumder, S., Biswas. A., &Rahman, M. M. (2016): *In vitro* mass propagation of *Gynura procumbens* (lour.) Merr. - an important medicinal plant. *Asian J. Natural & Applied Sci.* 5(3): 71-79.

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

Martin, K. P. (2003) Rapid *in vitro* multiplication and *ex vitro* rooting of *Rotulaaquatica*Lour., a rare rhoeophytic woody medicinal plant. *Plant Cell Rep.* 21:415-420.

Mathur, J.,&Mukunthakumar, S. (1996). Micropropagation of *Bauhinia veriegata* and *Parkensoniaaculeata* from nodal explants of mature trees. *Plant Cell Tissue OrganCul.* 28:169-175.

Mei, C. L. (2005). Micropropagation of *Vitisthunbergii*Sieb. et. Zucc., a medicinal herb, through high requency shoot tip culture. *Scientia Horticult*, 107: 64-69

Murashige, T.& Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*. 15: 473–497.

Nilanthi, D.,&Yang, Y. (2013) *In vitro* induction of octaploid from colchicine-treated tetraploid petiole explants of Purple Coneflower (*Echinacea purpurea* L.). *Tropical Agril. Res.Extn.*16.

Niranjan, M. H., Sudarshana, M. S.,&Girisha, S. T. (2010). *In vitro* multiple shoot induction from excised shoot tips and nodal segment explants of - *Lagerstroemia indica*(L) - A medicinal cum Ornamental Shrub. *J. Biomed. Sci. Res.*2(3):212-217.

Nowak, B., Miczynski,K., &Hudy, L. (2004). Sugar uptake and utilization during adventitious bud differentiation on *in vitro* leaf explant of WegierkaZwykla plum (*Prumusdomestica*). *Plant Cell Tissue Organ Cult.* 76: 255-260.

Parvin, F., Islam, M. J., Naoshin, J., Habiba, K., Shaekh, M. P. E., Aminul, I. M., Rahman, M. H., &Rahman, M. M. (2014). Efficient *in vitro* micropropagation of *Gynura procambens*- an important rate medicinal plant, throuth shoot tip and nodal segments. *J. Res. Biol*. 4(6): 1444-1450.

Rahman, M. M., Amin,M. N.,&Ahmed,R.(2004). *In vitro* rapid regeneration from cotyledon explant of native olive (*Elaeocarpusrobustus*Roxb.). *Asian J. Plant Sci.* 3: 31-35.

Rajeswari, V.,&Paliwal, K. (2008). *In vitro* plant regeneration of red sanders (*Pterocarpussantalinus*L.f.) from cotyledonary nodes. *Ind. J. Biotech*. 2:541-546.

Tiwari, K. N., Sharma, N. S., Tiwari, K.,& Singh, B. D. (2000). Micropropagation of *Centellaasiatica*(L.), a valuable medicinal herb. *Plant Cell Tissue Organ Cult.*63(3): 179-185

Tiwari, S. K., Kashyap, M. K., Ujjaini, M. M.,& Agrawal, A. P. (2002). *In vitro* propagation of *Lagerstromiaparviflora*Roxb. from adult tree. *Ind. J. Exp. Biol*. 40:212-215.

Xu, Z., Um, Y. C., Kim, C. H., Lu, G., Guo, D. P., Liu, H. L., Bah, A. A.& Mao, A. (2008). Effect of plant growth regulators, temperature and sucrose on shoot proliferation from the stem disc of Chinese jiaotou (*Allium chinense*) and *in vitro*bulblet formation. *Acta. Physiol. Plant*. 30:521–528.

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