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In vitro regeneration in *Syngonium* 'Mini Pixie' via protocorm like bodies

Priya Khetarpal* and Neelambika Meti

Department of Plant Biotechnology Rajiv Gandhi Institute of IT and Biotechnology, Bharati Vidyapeeth Deemed University, Pune-Satara Road, Katraj, Pune - 411046, Maharashtra, India

*Corresponding Author E-mail: priyakhetarpal@yahoo.in

ABSTRACT

Syngonium podophyllum'Mini Pixie' is an ornamental dwarf plant having green leaves with cream-white centers. Shoot tips of such plants were cultured on Murashige and Skoog's medium supplemented with individual and combined plant growth regulators such as Zeatin and Indole-3-acetic acid at 4.56 to 11.41μM concentrations. Clumps of PLBs were induced from the explants, on media containing combined growth regulators after twelve weeks of growth period. Subculture of PLBs on same medium or incorporating 1-Naphthalene acetic acid as an auxin, showed differentiation of stunted shoots both in dark and light conditions. Healthy plantlets were developed, when clumps of shoots were transferred to basal media without any plant growth regulators. Shoot multiplication from in vitro grown shoot tips was also observed on media supplemented with 1 mg/l of each 6-Benzylaminopurine and 1-Naphthalene acetic acid. The in vitro regenerated plants were harvested and maintained in closed glass containers with sand or stones and 20ml drinking water for making indoor decorative gift articles based on ornamental plants. This protocol of PLB induction, their germination, rapid shoot multiplication and plant regeneration can be applicable to the production of artificial seeds, micropropagation, transformation studies and also making innovative gifts.

Keywords*: Protocorm like bodies, Micropropagation, Regeneration, Syngonium 'mini pixie', Gift articles*

INTRODUCTION

Syngonium is commonly known as Arrow-head vine and belongs to the family Araceae. It has its origin at South and Central America[3]. As a result of their attractive foliar variegation and tolerance to low-light environments, cultivars from *S. podophyllum* in their juvenile stage have been widely produced as ornamental foliage plants and used as living specimens for interiorscaping[1]. The species "mini pixie" is a dwarf variety of *Syngonium* genus, its common name is African Evergreen.It is a perennial[6]plant and characterized by cream-white colored variegation at the center of its leaves. The characteristics of 'mini pixie' species make it more suitable as a foliage ornamental plant. It does not grow much in height and spreads around itself. This species makes a suitable indoor plant in places where space is a constraint. No work till now has been reported on this species of *Syngonium*. Use of micropropagation is favorable than conventional propagation as large numbers of plants are obtained in less space and time.

MATERIALS AND METHODS

Plant material

Shoot tips from mature mother plants were acquired from the potted plants growing in the Herbal garden of the Rajiv Gandhi Institute of IT and Biotechnology, Katraj, Pune, India. Dead or brown portions of explant were scraped off before carrying out surface sterilization.

Surface Sterilization

Explants were thoroughly rinsed under tap water for 30 minutes and then with distilled water. Treatment with 2% Bavistin for 15 minutes was done followed by thorough rinsing with distilled water. This was followed by a treatment with 2% Cetrimide for 15 minutes and distilled water. 2% Sodium hypochlorite treatment for 5 minutes was given. Finally, inside the laminar before inoculation,they were thoroughly rinsed4-5 times with sterilized distilled water.

Culture conditions and Media

The shoot tips were placed in culture vessels containing 50 ml of nutrient media with full strength MS basal salts with 3%sucrose, plant growth regulators such as Zeatin, Indole-acetic-acid, Benzyl amino acid, Naphthalene acetic acid, individually and in a few combinations. Media was solidified with 0.8% of agar. The pH was adjusted to 6 prior to autoclaving at 121 psi for 15 minutes.

Regeneration

For regeneration of PLBs into plantlets, two experiments were conducted for comparing the results and cost efficiency. In experiment 1, MS media was supplemented with Zeatin, NAA, or both. In experiment 2, media was supplemented with BAP and kept under dark and light conditions.

Shoot multiplication

Individual plantlets were transferred to MS medium supplemented with individual and combined BAP, NAA for shoot multiplication(Table IV).

Rooting

Rooting occurred in multiplication media and also in MS basal medium after shoot regeneration. Therefore, there was no need for subculture in media containing auxin for the establishment of plantlets.

***Ex vitro* transplantation**

For acclimatization, the shoots were treated with 2% Bavistin and then washed thoroughly with distilled water. Shoots were harvested in plastic cups containing cocopeat and the cups were placed in poly bags and closed tightly with rubber band to maintain humidity.Plants were kept in 16 hour photoperiod under cool luminescent lights at room temperature for 2 weeks.

All the harvested plants were provided with 5 ml of autoclave sterilized MS full strength salts A, B and C after transplantation and up to 2 weeks. Then plants were shifted to pots and maintained under greenhouse conditions.

Statistical analysis

12 explants were taken in one experiment and each experiment was repeated thrice. Thus, readings in tables are mean values of 12*3=36 for each combination of medium. Data was analyzed by one-way analysis of variance (ANOVA) ($P>0.05$), followed by Tukey-Kramer Multiple Comparison Test ($P>0.05$).

RESULTS

Induction of callus and PLB's

Shoot tips were inoculated on MS Basal medium supplemented with different combinations of Zeatin and IAA, and the results were shown in table I.

Callus induction was noticed after third week of culture and accompanied by protocorm like bodies on all media after 6 weeks of culture (Fig.1. B & C).However, best results were observed in media supplemented with 9.12µM Zeatin and 5.70µM IAA, with 91.67% cultures showing PLB formation. Protocorm-like bodies were hard globular green structures.

Regeneration

Experiment 1:

PLBs were cut into 1 to 1.5 cm pieces and inoculated on MS media supplemented with Zeatin and NAA, and the results are shown in table II.

After 4 weeks, root regeneration was accompanied with PLB's (Fig.1. D & E.) in all media containing Zeatin and NAA. However, best results were observed in media containing 4.56µM Zeatin and5.37µM NAA with 97.22% cultures showing response.

Experiment 2:

PLB's were inoculated on MS medium supplemented with BAP; each set was kept in dark and light conditions, as shown in Table III.After 4 weeks, stunted shoots along with callus proliferation was observed in both the sets, under light as well as dark conditions. Stunted shoots could not be easily distinguished from the callus. All the stunted shoots with callus obtained from experiment 1 and 2 were subculture on MS Basal medium (Fig1.F & G.), where the plant elongation with root regeneration was observed after 4 weeks.

Multiplication and rooting

Elongated *in vitro* plantlets were utilized for multiplication on MS supplemented with individual and combined BAP and NAA (Table IV).

Best results were observed in media containing 4.44µM BAP and 5.37µM NAA, where mean shoot length was found to be 9.00 cm and the mean number of shoots13.25.After 8 weeks, healthy plantlets were regenerated. Some of the roots were showing root hair.

Hardening and *Ex vitro* transplantation

Shoots harvested in cocopeat showed 100% survival rate after 4 weeks and then they were utilized for making gift articles. *Ex vitro* transplantation was also done in sand and multi-colored stones to obtain gift articles, which could be replacement for cut flowers for interior decoration. These gift articles are aesthetically more appealing and long lasting (Fig.1. H & I). These gift articles were supplied with only 50 ml of distilled water. They could last for more than eight months when covered with lid and provided proper light conditions at room temperature.

DISCUSSION

In the present study, callus was observed from the cut end of the 91.67% shoot tip explants on MS medium supplemented with 9.12µM Zeatin and 5.70µM IAA. Hard actively proliferating callus was identified as protocorm-like bodies. PLBs are distinguished from somatic embryos by the lack of a single embryonic axis[9]. Protocorm-like bodies were first documented by Morel[8] in *Cymbidium*, when the shoot apex was cultured, and later in other orchids[10,12]. PLBs are composed of many meristematic centers that are able to differentiate into shoots and roots[5]. Cui *et al.*[4] observed protocorm-like bodies in *Syngonium podophyllum* "white butterfly" on media supplemented with 2-iP, BAP, CPPU, or TDZ with 2, 4-D through nodal explants. Regeneration of PLBs was noticed on MS with PGRs Zeatin, NAA or both and individual BAP with stunted shoots that could not be distinguished from the callus. It was observed that shoot elongation was strongly inhibited by BAP in *Dracaena fragrans*[14]. Stunted shoots with PLBs were transferred to MS medium without any growth regulators. Shoot elongation and root regeneration was observed on MS basal medium. Shoot elongation was not observed on PGR containing medium, this could be explained by presence of hormone autonomy.Similar results were observed in *Anthuriumandraeanum*by Yi-xun *et al.*[15], where callus exhibited complete hormone autonomy for growth and differentiation of PLBs. The regenerated PLBs formed shoots and roots on ½ MS medium. Elongated plantlets were inoculated on multiplication medium, where best results were observed on MS with 4.44µM BAP and 5.37µM NAA. Mean shoot length was found to be 9.00 cm and the mean number of shoots was13.25.BAP and NAA was found to be suitable for multiplication in various plants such as in *Syngonium podophyllum*by Jiang *et al.*[7], *Cordyline* by Chinnu *et al.*,[2], *Dracaena* by Singh *et al.*[13]; *Spathiphyllum*for highest number of axillary shoots by Ramirez-Malagon *et al.*,[11].Special hardening procedure was not required; this was found to be similar to the results found in *Dracaena fragrans* by Vinterhalter D.V., in 1989[14]. 100% survival rate was observed in shoots harvested in cocopeat.

Table I. Response of callus induction from shoot tip explants after 6 weeks

PGRs (µM)		
Zeatin	**IAA**	**% of explants showing response**
0.00	0.00	0.00±0.00cdef
4.56	0.00	19.44±0.06de
9.12	0.00	22.22±0.07cd
0.00	5.68	11.11±0.05de
0.00	11.41	33.33±0.08cd
4.56	5.68	41.67±0.08bc
4.56	11.41	69.44±0.07ab
9.12	5.68	91.67±0.04a
9.12	11.41	77.78±0.07ab

Values represent percentage Mean± Standard Error(SE). Within the same column, values followed by the same letter are not significantly different (P>0.05).

Table II. Experiment 1 showing media used for regeneration of PLBs after 4 weeks

PGRs (µM)		Results	Response (%)
Zeatin	**NAA**		
0.00	0.00	Root regeneration	50.00±0.08d
2.28	0.00	Callus induction and PLB formation	33.33±0.08de
4.56	0.00	Callus induction and PLB formation	63.89±0.08cd
0.00	2.68	No response	0.00±0.00f
0.00	5.37	No response	0.00±0.00f
2.28	2.68	PLB's with stunted shoots	25.00±0.07def
2.28	5.37	----do----	44.44±0.08de
4.56	2.68	----do----	75.00±0.07bcd
4.56	5.37	----do----	97.22±0.02ab

Values represent percentage Mean± Standard Error (SE).Within the same column, values followed by the same letter are not significantly different (P>0.05).

Table III. Experiment 2 showing media used for regeneration of PLBs after 4 weeks

PGRs (µM)	Conditions	Results
BAP		
0.00	Under fluorescent light	Root regeneration
0.00	Under dark	Root regeneration
2.22	Under fluorescent light	Callus proliferation and stunted shoots
4.44	Under dark	Callus proliferation and stunted shoots

Table IV. Multiplication stage results observed after 8 weeks

PGRs (µM)		Response	Mean shoot length (in cms.)	Mean number of shoots per explant
BAP	NAA			
0.00	0.00	Regeneration of roots	0.00±0.00e	0.00±0.00f
2.22	0.00	Multiple shoots	2.41±0.18d	0.00±0.00f
4.44	0.00	Multiple shoots	2.25±0.14d	0.00±0.00f
0.00	2.68	Shoot elongation	3.00±0.13d	2.00±0.15e
0.00	5.37	Shoot elongation	3.58±0.13d	2.50±0.13e
2.22	2.68	Multiple shoots	4.50±0.15cd	4.00±0.17d
2.22	5.37	Multiple shoots	8.00±0.26b	5.94±0.23c
4.44	2.68	Multiple shoots with roots	7.50±0.24b	8.50±0.21b
4.44	5.37	Multiple shoots with roots	9.00±0.27ab	13.25±0.21a

Values represent the Mean ± Standard Error (SE).Within the same column, values followed by the same letter are not significantly different (P>0.05).

Graph I. Rate of shoot multiplication and length of shoots

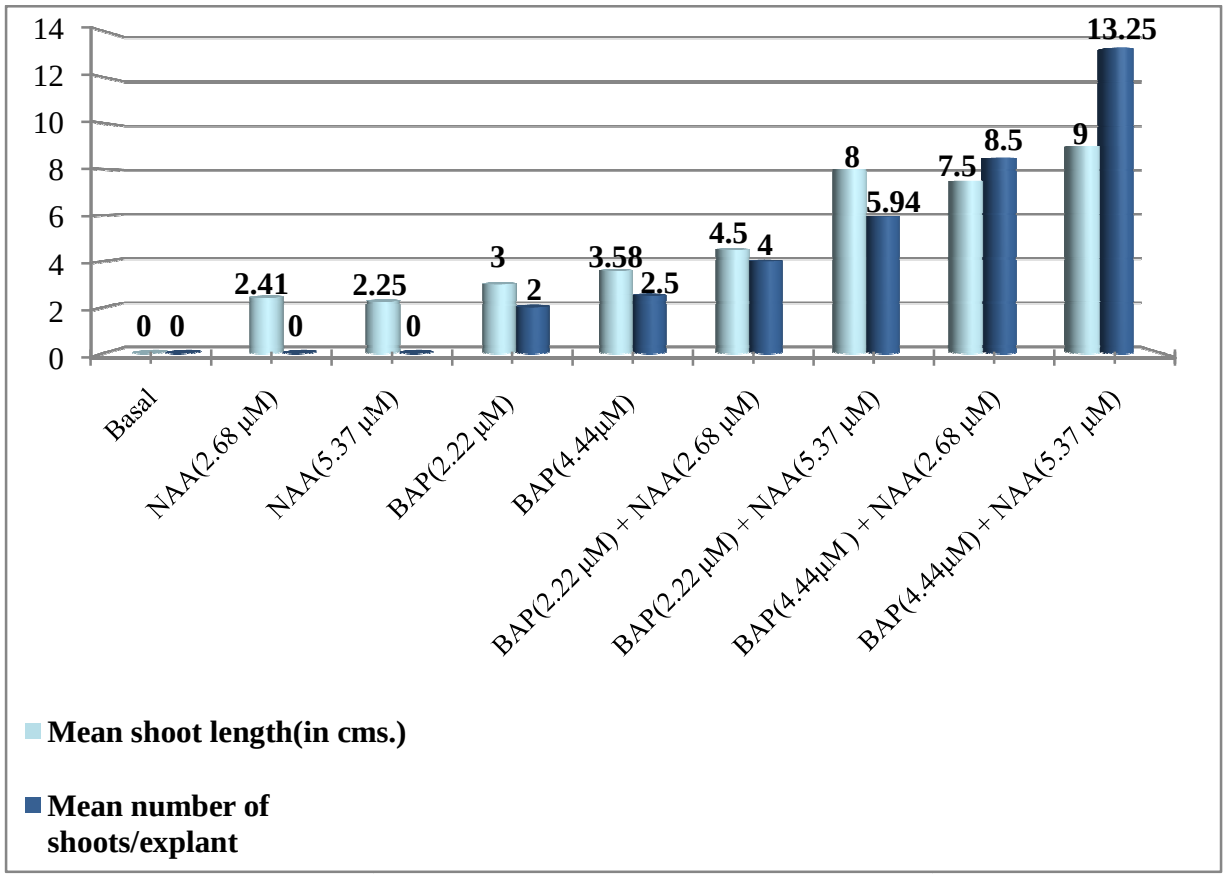
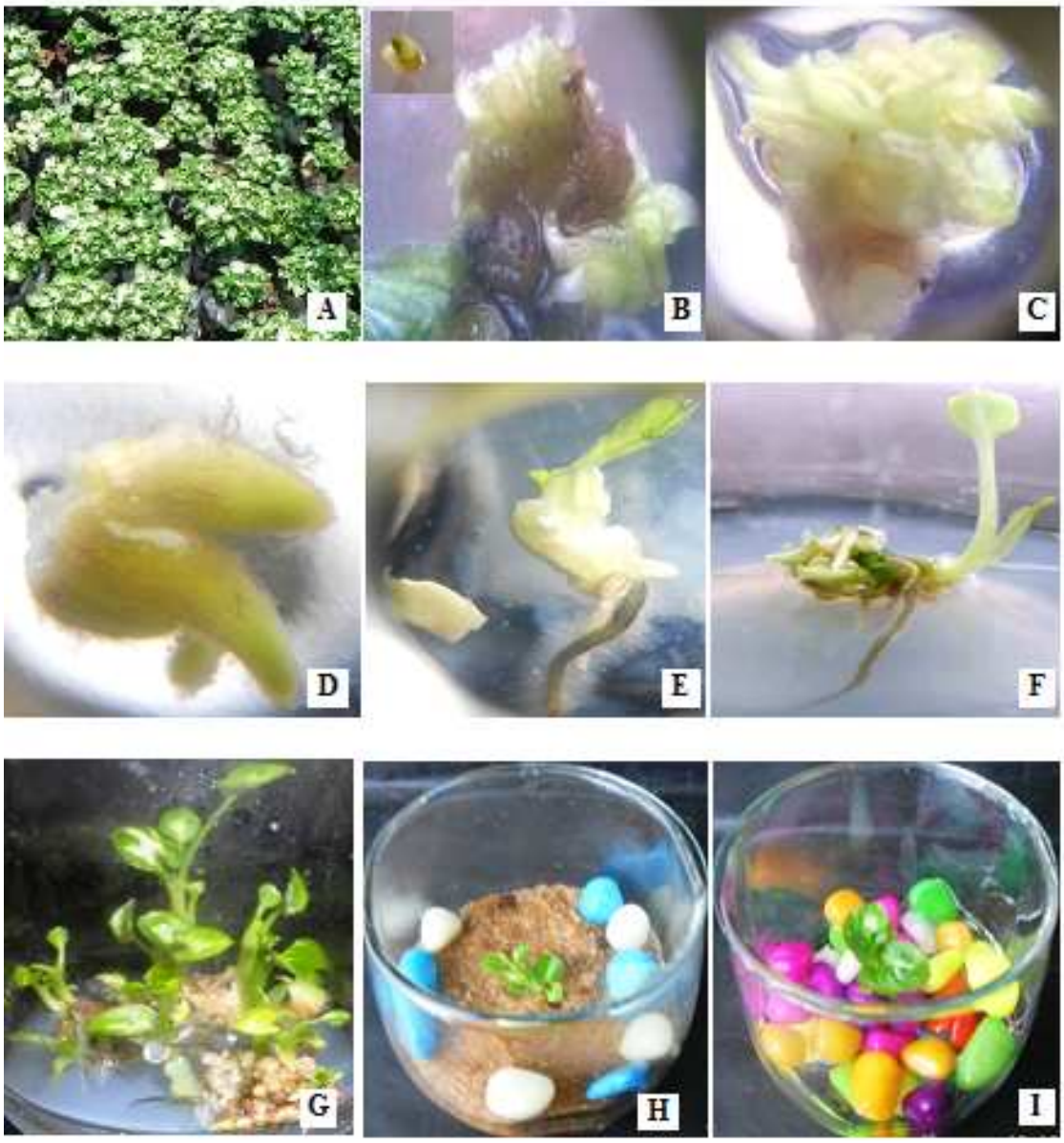


Fig: 1 *In vitro* plant regeneration in *Syngonium 'Mini Pixie '* via PLB's



A)Mother plants in polybags B) 12wks old shoot tip culture showing protocorm like bodies (PLBs) C) Clump of PLBs D&E) Germinating PLBs F) Regeneration of plantlet G) PLBs and regenerated plants ready for harvesting H & I) Harvested plants growing in covered glass vessels with sand and stones.

CONCLUSION

A protocol has been established for *in vitro* regeneration and multiplication of *S. podophyllum* 'mini pixie' from shoot tip explants via indirect organogenesis for rapid micropropagation. For callus induction, MS with 9.12µM Zeatin and 5.70µM IAA was found to give best results. In our opinion, 2.22µM BAP is suitable for inducing stunted shoots as it will be cost effective as compared to use of Zeatin and NAA, since both of the experiments gave same results. Further, for multiplication 4.44µM BAP and 5.37µM NAA gave best results. Gift articles were made from regenerated plantlets. These gift articles are aesthetically more appealing, long lasting and low maintenance, supplied with only distilled water and therefore, as a whole can be replacement for cut flowers and bouquet. These gift articles could last for more than eight months when provided proper light conditions at room temperature.

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