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Research Article

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In vitro Micropropagation of *Syngonium podophyllum*

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ABSTRACT

Micropropagation of Syngonium podophyllum was performed using axillary shoots as explants. Initiation of shoots and multiplication of shoots could be induced on Murashige and Skoog (MS) medium supplemented with BAP + NaH_2PO_4 (4.44 µM +200 mg/l). This combination induced 17.33 shoots with the maximum shoot length of 7.3cm. The root induction was observed on the same medium or when the shoots were transfer to the same medium with the percentage of 96.66 and root length of 10.12cm. Acclimatization was performed successfully with 97% survival rate on commercially available sterile potting mix in net pots and hardened in a shade house under 90-95% relative humidity for 10-20 days followed by transfer to soil.

Key words: *Micropropagation, Syngonium podophyllum, Acclimatization.*

INTRODUCTION

Syngonium podophyllum Schott, commonly known as arrowhead vine, goosefoot plant, arrowhead wine or nephthytis, belongs to the family Araceae and occurs indigenously on humid forest floors of Central and South America[1]. 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 interiors caping[2]. Eye cuttings, arrowhead vine has been micropropagated using shoot tips[3]. Abuot 19 million plantlets of arrowhead vine are annually micropropagated worldwide, which has greatly reduced the incidence of diseases carried by eye cuttings. *Myrothecium* leaf spot disease is the one of the most common fungal disease caused by *Myrothecium radium.* Their air borne fungal pathogen occurs during the *ex vitro* rooting of micro cutting because the cutting base is especially susceptible to this pathogen[4].

Replacing *ex vitro* rooting with *ex vitro* transplanting should offer a solution to controlling *Myrothecium* leaf spot in arrowhead vine. Micro cutting should be rooted *in vitro* and then transplanted *ex vitro.* Another solution could be the regeneration through a simple reproducible commercially viable one step protocol for initiation, multiplication and rooting. In which well rooted plantlets could be produced for *ex vitro* transplanting.

In this study we developed an efficient, reproducible, low cost, one step protocol for regeneration of disease free, well-rooted plantlets of *S. podophyllum* "pink" for *ex vitro* transplanting.

MATERIALS AND METHODS

Plant material

Stem cuttings including the youngest leaf were excised from one year old *S. podophyllum* "pink" stock plant grown in a shade house condition at Government Arts college, Coimbatore. Leaves and petiole were excised from stem section defoliated stems with nodal buds were surface sterilized by immersing in 70% ethanol for 30 sec and soaking in a 0.12% mercuric chloride ($HgCl_2$) solution for 4min. After pouring off the $HgCl_2$ solution, the explants were rinsed three times with sterile distilled water.

Murashige and Skoog mineral salt and vitamins[5] with 3.0% (w/v) sucrose and 0.8% (w/v) agar (Hi media) were used as a basal medium. The pH of the medium was adjusted 5.8 with 1M NaOH before autoclaving at 121ºC for 20 min. Plant growth regulators were added before autoclaving. Three different type of growth regulators combination and concentrations were used for regenerating plantlets from nodal explants under the light conditions. Nodal explants were cultured on the basal medium supplemented with BAP (2.22 – 13.32µM) + NaH_2PO_4 (200 mg/l) or BAP (2.22 – 13.32µM) with NAA (2.14µM) or BAP (2.22 – 13.32µM) with TDZ (0.90µM).

Sterilized nodes were cut into one cm long segments in a sterile culture bottles. The explants were transferred onto cultured bottle containing 30 ml of basal medium supplemented with the above mentioned growth regulators. There were four nodal explants per bottle and the experiment was repeated three times.

Data collection and analysis

A complete random design was used for the three experiments. Each bottle was considered as experimental unit, and each treatment had six replications. Explants that responded to the induction were recorded per bottle from 4 to 15 weeks after culture, and frequencies of the response were calculated.

Root development and *ex vitro* plantlets establishment

The root formation occur on the same medium (BAP+ NAA) or the well-developed shoots were separated and transferred to the same medium. Regenerated plantlets were separated, washed free of agar using tap water, and transplanted into a hardening media consisting of decomposed coir waste, perlite and compost in a 1:1:1 ratio based on volume. Potted plants were grown in a shade house under the temperature range of 25 to 28º C, and relative humidity of 70% to 90%. Survived rate of plantlets in the shade house were recorded 45 days after transplanting.

RESULTS

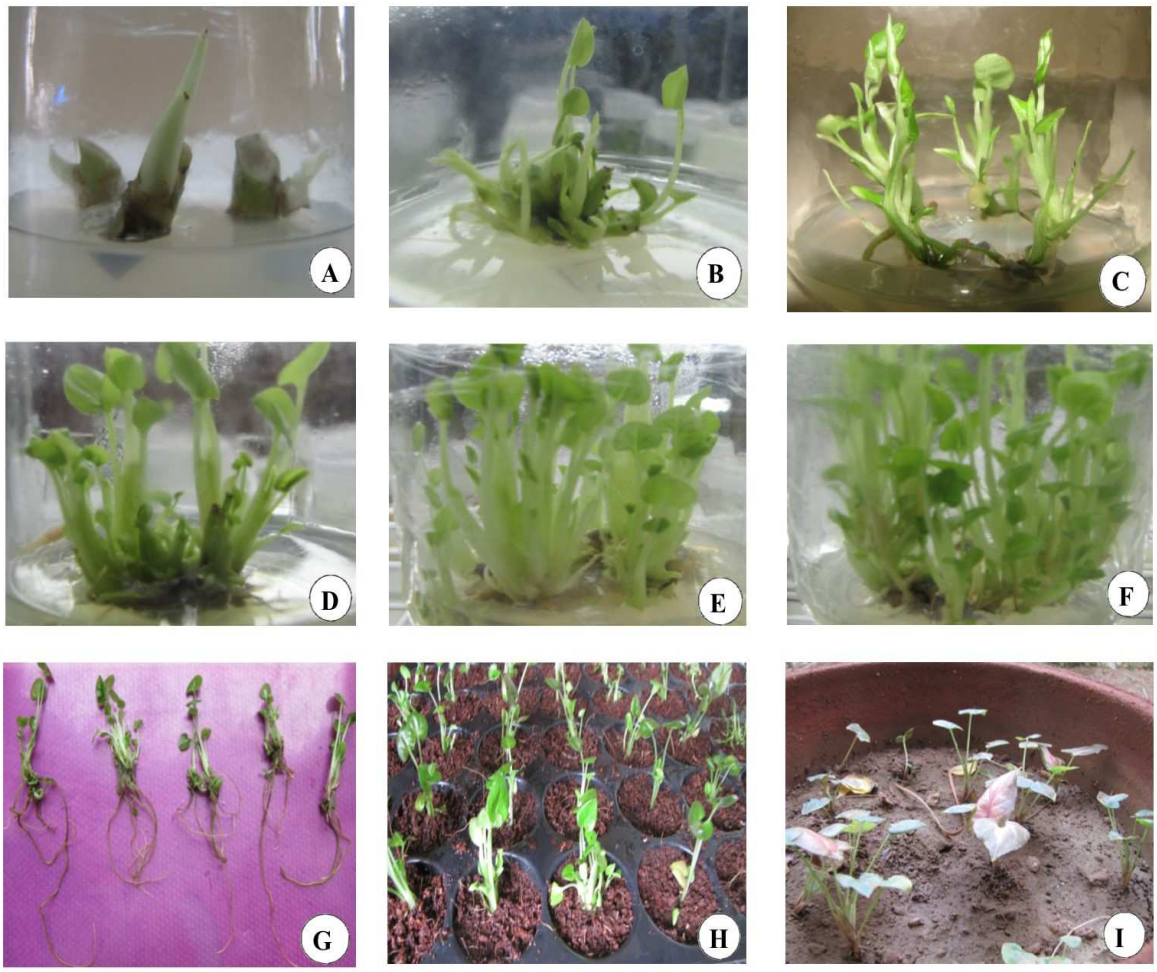
Explants numbering 20 were inoculated on MS medium with the combination of BAP and NaH_2PO_4, BAP and NAA, BAP and TDZ. Among the various treatments, effective results were obtained from combinations given in Table 1. Explants cultured on all the media induced one or two shoots within 6-8 days. Shoot proliferation was best in BAP + NaH_2PO_4 (4.44 µM +200 mg/l), (Figure 1 E and F) followed by another combination BAP+TDZ (4.44 µM + 0.90 µM) 20 days after subculture. Other combination of MS with BAP+NAA, MS with BAP +TDZ and BAP+ NaH_2PO_4 were also effective, but not at the level of the previous combinations. Sub culturing of the shoots for multiplication on the same medium (BAP + NH_2PO_4) induced multiple shoots. After two or three subcultures clump formation occurs. The proliferating axillary buds were well defined, pale green and 0.5 to 1.0 cm long. A threefold increase in multiplication was seen by 4-5 weeks. Further transfer with same medium regulated in three to four fold ratio at every sub culture cycle.

Explant cultured for 8-10 weeks in MS, BAP + NaH_2PO_4 (4.44 µM +200 mg/l), medium which had reached the stage of vigorous proliferation, consisting of 17 .33 ± 1.30 shoots with the maximum shoot length of 7.3±0.81 were divided into small clumps (Figure 1 E and F). Each clump consisting of 4-6 shoots was transferred to the same medium. On the same medium dwarf shoots recorded to normal growth with more number of axillary shoots. At the same time 5-6 vigorously growing shoots from each clump elongated with expanded leaves by three to four weeks (Figure 1). These shoots had healthy transfer in the same medium and gave rise to a four to five fold increase in proliferating clumps and 5-6 elongated shoots with roots initials from each clump (Figure 1 G). The elongated shoots were excised and cultured separately in the same fresh medium to encourage formation of long shoots, broad leaves and basal roots. Basal tufts of rooting were observed in 96.66 ±2.80 % of the transferred shoots with the root length of 10.12±0.52 cm. The elongated shoots with roots were transferred shoots washed thoroughly in running tap water and planted in hardening media, a commercially available sterile potting mix in net pots and hardened in a shade house under 90-95% relative humidity for 10-20 days. They were gradually transferred to plastic pots or polythene bags (Figure 1H and I). A survival rate of 97% was achieved during the hardening.

Table-1 Effect of BAP, NAA, TDZ and NaH_2PO_4 on initiation, multiplication and rooting from nodal explant of *S. podophyllum* on MS medium

S.No	BAP (µM)	NAA (µM)	TDZ (µM)	NaH_2PO_4 (mg/L)	Shoot no per explant	Shoot no per explant/Sub culture	Shoot length (cm)	Rooting %	Root length (cm)
1	2.22	-	-	200	1	10.33±1.63	5.16±1.47	71.16±1.94	7.95±0.53
2	**4.44**	**-**	**-**	**200**	**2**	**17.33±1.03**	**7.33±0.81**	**96.66±2.80**	**10.12±0.52**
3	6.66	-	-	200	2	09.00±.1.41	6.26±1.23	75.83±3.31	6.18±0.31
4	8.88	-	-	200	1	07.50±1.40	5..40±0.54	60.16±3.86	6.08±0.36
5	11.10	-	-	200	1	03.16±1.16	4.80±0.59	-	-
6	13.32	-	-	200	1	02.16±0.75	3.08±0.51	-	-
1	2.22	2.14	-	-	1	05.48±0.75	4.56±0.50	21.16±2.63	3.13±0.25
2	4.44	2.14	-	-	1	07.43±1.03	3.11±0.41	24.83±1.47	4.21±0.60
3	6.66	2.14	-	-	1	04.16±0.75	4.40±0.56	10.50±1.04	2.10±0.36
4	8.88	2.14	-	-	1	03.66±0.81	5.01±0.87	05.00±0.89	2.01±0.23
5	11.10	2.14	-	-	1	02.16±1.16	3.05±0.72	-	-
6	13.32	2.14	-	-	1	02.16±1.47	4.13±1.33	-	-
1	2.22	-	0.90	-	2	06.83±1.13	6.15±0.86	50.66±3.66	3.90±0.61
2	4.44	-	0.90	-	2	13.83±1.85	7.07±0.42	70.33±3.07	6.13±0.45
3	6.66	-	0.90	-	1	08.33±1.21	5.53±.081	60.00±2.36	8.03±0.45
4	8.88	-	0.90	-	1	07.33±2.16	3.98±0.54	21.00.±2.80	5.96±0.39
5	11.10	-	0.90	-	1	03.83±1.47	3.01±0.52	10.33±1.63	4.80±0.61
6	13.32	-	0.90	-	1	03.0±1.09	4.10±0.58	-	-
	MS BASAL	-	-	-	-	-	-	-	-

Plate –1 *Syngonium podophyllum* A. &B Initiation of Shoots from node. C&D Clump formation E&F Multiple shoot formation G. Rooted plants H. Primary Hardening I. Secondary Hardening



DISCUSSION

Floriculture puts its best foot forward to shift a considerable amount of capital in productive change every year[6]. Meristem culture allows rapid multiplication and virus elimination for the study of shoot and root development[7,8]. The results of present study revealed that nodal explant cultured on MS medium substituted with the combination of BAP and NaH_2PO_4, BAP and NAA, BAP and TDZ induced the shoot induction response. Shoot proliferation was best in BAP + NaH_2PO_4 (4.44 µM +200 mg/l). By increase or decrease in BAP concentration, the role of shoot formation was decreased. Aamir Ali *et al.* (2007)[9]also reported that the best shoot formation response in MS medium supplemented with BAP in *Caladium bicolar.* TDZ is a cytokinin like compound that can promote shoot proliferation[10]. According to Akasaka *et al.* (2000) [11] TDZ is the more efficient than the BAP, Zeatin and kinetin. Yeh *et al.* (2007) [12] also used TDZ combined with dicamba in the tissue culture of *Aglaonema.* But in the contrary, in the present study BAP with NaH_2PO_4 combination were more efficient than the BAP and TDZ combination.

This study was intended to established an efficient method for regenerating well rooted plantlets of *S. podophyllum* 'pink' through nodal explants.

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