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Research Article

IN VITRO PROPAGATION OF *SPATHIPHYLLUM FLORIBUNDUM (L) 'PETITE'*

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ABSTRACT
Simplified method of *in vitro* propagation of *Spathiphyllum floribundum (L) 'Petite'* for the mass propagation has been developed. The ex-plants were collected from the well developed true to type mother plants. The explants were trimmed to the size of 1 to 2 cm, followed by tap water wash for 30 minutes. Again it was treated with tween -20 with antifungal solution for another 30 minutes, followed by tap water wash until the solution became cleared. Then, again it was treated with 20 % sodium hypochlorite with few drops of tween- 20, followed by washed with double distilled water four to five times. Finally at last the explants were taken into the laminar flow bench & treated with 1 % mercuric chloride for 1 minute followed by wash with Double Distilled water four to five times. After the proper sterilization the cultures were inoculated in establishment media with the combination of, Basal MS medium with BAP- 0.5 mg/l + 0.1mg/l IAA+ sugar 30 gram+ agar 6gm. After four weeks the successful cultures were transferred to the shoot multiplication & differentiation medium with different combinations. Out of another five trails, MS basal Medium+ BAP, 2mg/l + IAA, 1mg/l + sucrose 30gm/l+ agar, 6gm/l., was found the best. It was evaluated that the same combination of medium was suitable for both shoot multiplication and the rooting. Finally, well elongated rooted shoots were taken for the Acclimatization. For primary hardening the plants were kept in 90% humidity for one month in the Portray filled with coco peat. After the successful Primary Hardening the plants were transferred to the secondary hardening poly bag with different substrate combinations, out of them (Coco peat +Sand +Vermicompost) in the ratio of 2:1:1 was found the best. The plants were successfully hardened in 1.5 month with well developed shoots & roots.

Keywords: MS- *Murashige & Skoog, BAP- 6- Benzyle Amino Purine, IAA- Indole Acetic Acid, Micropropagation etc*

INTRODUCTION
Spathiphyllum floribundum (L) 'Petite' is one of the well known ornamental pot plant. It is peculiar for its unique sapth. It belongs to the Araceae family. It is shade loving perennial plant. It has clusters of leaves that grow directly from rhizome shoot. It is also propagated through rhizome propagation. The leaves are dark green on either side. The blooming of flowers generally occurs during the onset of spring, which last long. It is globally distributed mainly in warm, humid, and tropical forest of Asian content. The demand of the plant is enhanced globally & it becomes the main pot plant for the major import & export (Rout *et al.*, 2006).
It can be propagated by both asexual and sexual methods. But the propagation by the conventional methods is not commercially viable to fulfill the growing demands of quality planting material day by day. It is essentially required to develop the commercially viable and fast method of propagation to fulfill the demand of planting material globally to capture the export market. No doubt the *In vitro* micro propagation is one of the best methods which only can make it possible in shorter period of time & under controlled lab condition throughout the year with consistent quality & quantity.

MATERIALS AND METHODS
The explants were collected from the disease free true to type potted mother plants under green house condition. The mother plants were treated intermittently with antifungal & anti bacterial foliar spray for

two month before the collection of explants. The rhizome meristem were collected as explants, after proper trimming the explants were forcefully washed in tap running water for 30 minutes to remove all the soil debris.

It was trimmed to the size of 1cm to 2cm, again washed with few drops of tween -20 for 30 minutes followed by multiple wash in tap water. For the further process the explants were taken in sterile area of lab under the laminar flow bench, then again the explants were treated with 20 % sodium hypochlorite with few drops of tween -20, as advocated earlier by Diniz *et al.*, (2008), followed by five times washed in double distilled water.

Finally explants were treated with 0.3% acidified mercuric chloride, for 5 minutes, followed by washed in 4 to 5 times sterilized double distilled water. After proper sterilization the cultures were trimmed to the appropriate size to remove the outermost damaged cells & were inoculated in Basal MS medium.

Medium Preparation

The Basal Medium (Murashige & Skoog medium, 1962) was prepared with different concentration of Growth regulators, as it is recommended earlier by Dewir *et al.*, (2006) under the temperature of 121 degree centigrade, for 15 psi, for 15 minutes duration. The ph was adjusted to 5.7 with 1N HCL & I N KOH.

Establishment of Cultures

The cultures were inoculated in MS basal Medium+ BAP 0.5 mg/l+ IAA 0.1 mg/l+ Agar 6 gm/l+ sucrose 30 gm/l. The cultures were incubated in 12 hours photoperiod with relative humidity 60 %, temperature ranging from 25 to 27 degree centigrade. The duration given for incubation was 4 to 5 weeks.

Cultures Multiplication & Rooting

For the Multiplication & rooting of cultures different five trails of the medium were done. In all the trails the basic MS medium was taken with varying concentration of the BAP ranging from (0.5 mg/l, 1 mg/l, 1.5mg/l, 2mg/l, 2.5mg/l) as used earlier by Das *et al.*, (2000) & IAA, (0.05mg/l, 0.15mg/l, 0.1mg/l, 0.2mg/l, 0.25mg/l) as used earlier by Malagon *et al.*, (2001)+ Agar 6gm/l+ Sucrose 30gm/l. The cultures were incubated in 12 hours photoperiod with relative humidity 60 %, temperature ranging from 25 to 27 degree centigrade. Most of the authors advocated previously for the use of higher concentration of cytokinin and auxin for the shoot multiplication stages (Malagon *et al.*, 2001; Dewir *et al.*, 2006).

Acclimatization

The well grown healthy rooted shoots were taken for the acclimatization. The shoots were taken for primary hardening in 98 cavity portray filled with coco peat. The potted plants in portray, were kept in 98% humidity for one month duration. The two stage hardening was also advocated earlier by Van Huylenbroek and De Riek (1995). During the time of primary hardening, the antifungal solution was sprayed intermittently.

For the secondary hardening, the four trails were conducted to find out the most suitable potting material for the efficient growth during the secondary hardening of the plants. In trails HI– Coco peat+ Vermiculite+ soil in ratio (1:1:1). In case of trail code H2. Coco peat +soil+ Vermi compost in the ration of (1:1:2). In case of H3 – soil+ cow dung, (1:1). In trail H4, cocopeat+ soil+ vermiculite, in the ration of (2:1:1). The duration given for the secondary hardening was two month. Mild fertilizers were applied to enhance the growth.

RESULTS AND DISCUSSION

Multiplication & Rooting

The five trails of Multiplication & rooting were evaluated. In all the trails BAP & IAA were used in different concentrations.

The best response was found in the Trail code T4 (MS medium+ BAP, 2mg/l+ 0.2 mg/l IAA+ agar 6 gm/l+ sucrose 30 gm/l.), it gave excellent multiplication ratio. Each trail was conducted twice with 5 replication to establish the authenticity of result. It gave 8.2 nos of Mean healthy shoots with 95% healthy roots. It was also noticed that the same medium was suitable for both shoot multiplication & rooting plants in five weeks duration.

Table 1: Multiplication & Rooting Trails

Trail Code	Medium	BAP (mg/l)	IAA (mg/l)	No shoots Mean ± SE	% of Healthy Roots
T1	**MS**	**0.5**	**0.05**	**3.2 ± 0.17**	**23**
T2	**MS**	**1**	**0.1**	**4.8 ± 0.42**	**45**
T3	**MS**	**1.5**	**0.15**	**6.4± 0.35**	**45**
T4	**MS**	**2**	**0.2**	**8.2 ± 0.33**	**95**
T5	**MS**	**2.5**	**0.25**	**4.4± 0.21**	**45**

* (5) Five replication were used for each treatment and all the experimental trails repeated twice.

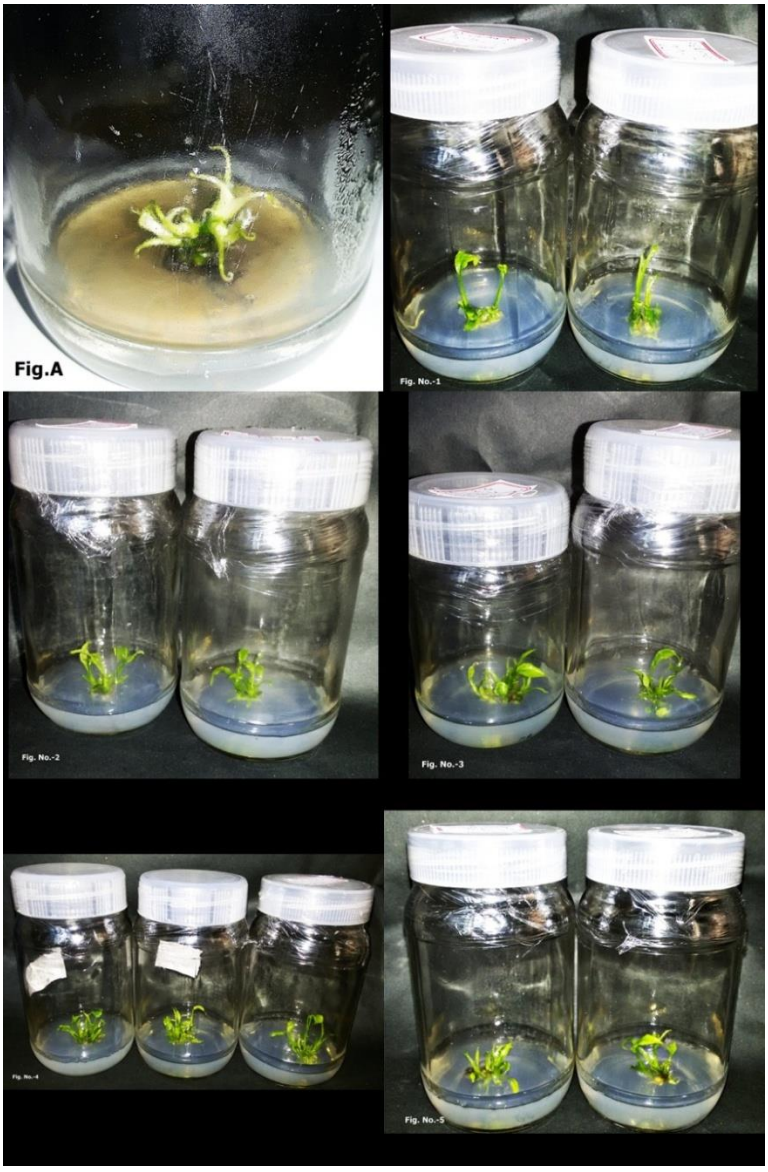

Figure A: Initiation of Cultures Shoots Not Differentiated after Three Weeks; Figure 1: Trail T1 (MS+ BA, 0.5 mg/l + IAA, 0.05 mg/l); Figure 2: Trail T2 (MS+ BA, 1 mg/l + IAA, 0.1 mg/l); Figure 3: Trail T3 (MS+ BA, 1.5 mg/l + IAA, 0.15 mg/l); Figure 4: Trail T4 (MS+ BA, 2 mg/l + IAA, 0.2 mg/l); Figure 5: Trail T5 (MS+ BA, 2.5 mg/l + IAA, 0.25 mg/l)

Acclimatization

For the hardening of cultures two stage hardening was evaluated. In case of primary hardening the rooted cultures were taken outside the lab, graded properly, & were washed to remove the traces of the agar & were treated with antifungal solution. Then, plants were transplanted in Portray filled with coco peat, & were kept in 98 % humidity for one month. Intermittent fertilizers were given to enhance the growth.

In case of secondary hardening four trails was done among them the trail code H4 (Cocopeat+ Sand+ Vermiculite) in the ratio of (2:1:1), was found the best one which resulted well elongated 90 % hardened plants in 1.5 months with well developed morphology.

Table 2: Hardening Trails

Trails	Substrate used	Ratio	Fertilizers used	% of Healthy Plants
H1	Cocpeat+Vermiculite+Soil	1:1:1	½ MS spray	78
H2	Cocopeat+Soil+Vermi	1:1:2	"	36
H3	Sand+Cowdung	1:1	"	60
H4	Cocopet +Sand+V.culite	2:1:1	"	90

Figure 1: Cocopeat+ Vermiculite+ Soil (1:1:1); Figure 2: Cocopeat+ Soil+ Vermicompost (1:1:2); Figure 3: Sand+ Cowdung (1:1); Figure 4: Cocopeat+ Sand+ Vermiculite (2:1:1)

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We hereby acknowledged that the above work is sole work of us under lab condition**.** It was found that the above observation is very helpful for the mass propagation for the Spathyphyllum. The large no of healthy plants can be produced in short duration of time.

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