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Response of Growth Hormones on Staghorn fern (*Platycerium bifurcatum* (Cav.) C.Chr.) Gametophytes under *in vitro* Condition

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ABSTRACT: Staghorn fern is an ornamental fern which is epiphytic in nature and reproduce through transformation of generation, which happens over a long time. The present study aims to reduce the transformation phase of gametophyte to sporophyte by the influence of growth hormones under *in vitro* condition. The gametophytes of staghorn fern (*Platycerium bifurcatum* (Cav.) C. Chr.) were subjected to three different growth hormones such as auxin, gibberlin and cytokinin at different concentrations to enhance the transformation in short time. The results revealed that, the treatment with auxin at 1.0 mg l^{-1} BAP resulted in the higher transformation of gametophytes to sporophyte, but the cytokine in treated were further proliferated, without any transformation. However, gibberlins at 3.0 mg l^{-1} was found to be better with respect to broader leaves with taller sporophytes.

Keywords: *Platycerium bifurcatum* (Cav.) C.Chr., Staghorn fern, gametophyte, sporophyte, auxin, gibberlin, cytokinin.

INTRODUCTION

The Staghorn is an ornamental fern belonging to the family polypodiacea. It's an epiphytic in nature, consisting of 15-18 species, cultivated worldwide because of its appearance (Hoshizaki, 1972). These plants are distinguished from other ferns by their characteristics and differentiation of the leaves into base fronds or mantle leaves and forked fertile leaves which are unique in nature, so they have an important place in floriculture and landscaping. They have a great economic value which cost around Rs. 200-500 per plant based on the age.

The ferns possess a special reproductive system known as "alternation of generation" where, a spore germinates to produce gametophyte which contains both reproductive organs such as antheridium and archegonium, upon fertilization sporophyte emerges out and the gametophyte gets degenerated. They are conventionally propagated by either spores or pups. It is known that the conventional propagation of ferns from spores is slow, while vegetative propagation is also hampered by low multiplication rate. So the *in vitro* propagation is an important tool to overcome the limitations. A variety of methods for *in vitro* vegetative propagation of several species in this genus has been successfully studied, including direct sporophyte regeneration from the shoot tip (Hennen and Sheehan, 1978), homogenized leaf tissue (Cooke, 1979; Teng and Teng, 1997), entire leaf explants (Camloh and Gogala, 1991; Camloh *et al*., 1994), rhizomes (Wee *et al*., 1992) and bud scales (Ambrozic-Dolinsek and Camloh, 1997). The duration of the process from spore to sporophyte can vary from 1 to 8 months in different ferns (Fernandez *et al*. 1999). In *Platycerium* species, the length of this process, determined by using *in vitro* culture of spores, varies from 3 months in *P. bifurcatum* (Camloh *et al*., 2001), and 5 months in *P. ridleyi* (Rodpradit, 2003) to 7 months in *P. grande* (Amoroso and Amoroso, 2003). The effects of exogenous treatment with cytokinins, auxins, gibberellins, ethylene, jasmonic acid, and brassinosteroids on spore germination, gametophyte morphology and development are analysed (Romanenko *et al.*, 2020). The pattern of gibberellic acid and zeatinriboside accumulation has shown the key role of these hormones in the regulation of growth processes and the development of reproductive structures of *P. aculeatum* gametophytes (Kosakivska *et al.*, 2020). Even though there is a lot of scope for development of protocol in regeneration system, no recent findings have been reported in the particular species. The present study aims to reduce the transformation phase of

gametophyte to sporophyte by the influence of growth hormones under *in vitro* condition which can be helpful produce large number of plants in shorter span of time.

MATERIAL AND METHOD

The study was undertaken during the year 2016-17 at the Plant Tissue Culture Laboratory, Division of Horticulture, University of Agricultural Sciences, Bangalore, GKVK, Bengaluru. The *in vitro* grown three month old gametophytes were taken for the experiment, the clumps are taken out from the culture bottles and separated out, inoculated on the basal MS media containing indole-3-butyric acid (IBA), 6-benzyl-amino-purine (BAP) and gibberellic acid (GA_3) at 0.5, 1.0, 1.5, 2.0 and 3.0 $mgl^{-1}$with control. Each operation was carried out in ascetic condition under laminar air flow chamber (LAF). The culture transferred bottles were kept in growth room having temperature of 24 ± 2°C. Light intensity of 2000 lux was provided using white fluorescence tubes for eight hours of light and 16 hours dark period.

The observations were recorded at fortnight interval from the day of inoculation. Each treatment was replicated ten times. Analysis of variance estimated by adopting complete randomized design. The test of significance was observed at one percent.

RESULTS AND DISCUSSION

Days took for the emergence of the sporophyte: The data on the mean number of days taken for the emergence of sporophyte is presented here (Table 1). Significant differences were observed in reference to the effect of growth regulators on days taken for sporophyte development from gametophytes. Significantly least number of days (25.30) was taken for the emergence of sporophyte in gametophytes cultured in the media containing 1 mg l^{-1} BAP. The emergence of sporophyte from the gametophyte took more number of days with increasing concentration of BAP in the media. This concentration appears optimal for early production of sporophyte from the gametophytes cultured. It's evident from the observation that gametophytes require lower amount of BAP at juvenile phase for early emergence. Similar results of advanced emergence of sporophyte from cultured gametophyte was reported by Melan and Whittier (2010); Kwa *et al*., (1995) in *Platycerium bifurcatum*.

Table 1: Effect of growth hormones on emergence and length of sporophytes.

Treatments	Mean no. of days taken for the emergence	Mean length(cm.)
Basal MS + 0.5 mg l^{-1} BAP	27.20	1.75
Basal MS + 1.0 mg l^{-1} BAP	25.30	2.00
Basal MS + 1.5 mg l^{-1} BAP	28.40	1.76
Basal MS + 2.0 mg l^{-1} BAP	28.80	1.70
Basal MS + 3.0 mg l^{-1} BAP	31.00	1.22
Basal MS + 0.5 mg l^{-1} IBA	32.20	1.02
Basal MS + 1.0 mg l^{-1} IBA	31.00	0.92
Basal MS + 1.5 mg l^{-1} IBA	29.60	0.89
Basal MS + 2.0 mg l^{-1} IBA	31.20	0.85
Basal MS + 3.0 mg l^{-1} IBA	33.40	0.71
Basal MS + 0.5 mg l^{-1} GA_3	34.40	2.20
Basal MS + 1.0 mg l^{-1} GA_3	32.60	2.56
Basal MS + 1.5 mg l^{-1} GA_3	33.20	3.01
Basal MS + 2.0 mg l^{-1} GA_3	34.20	3.56
Basal MS + 3.0 mg l^{-1} GA_3	35.10	2.75
Basal MS (Control))	30.40	2.55
F-test	**	**
SE.m±	0.45	0.12
CD	1.53	0.34

Number of sporophytes emerged: The data on mean number of sporophytes emerged are presented here (Table 2). The data recorded after 30 days of culture of gametophytes (Fig. 1) did not show any significance response towards emergence of sporophytes. While the observations made after 45 days at regular fortnightly intervals up to 150 days of culture were found to be significant in response of gametophytes to number of sporophyte. After 45 days of culture, the mean number of sporophyte emerged was significantly higher in basal MS media (Fig. 2), the data recorded with 1.5 mg l^{-1} BAP was on par with each other, while the lowest number was recorded in the media with 3 mg l^{-1} GA_3 (0.26). Similar results were obtained as on 60 days of culture (Fig. 2). The media supplemented with basal MS media (1.75) was found to be significantly different which is also on par with the media supplemented with 1 mg l^{-1} BAP (1.23) and 1.5 mg l^{-1} BAP (1.27).

Table 2: Effect of growth hormones on sporophyte production.

	Days after sub-culture				
Treatment	**30**	**60**	**90**	**120**	**150**
Basal MS + 0.5 mg l^{-1} BAP	0.40	0.86	1.63	2.10	2.79
Basal MS + 1.0 mg l^{-1} BAP	0.91	1.23	1.79	2.55	2.80
Basal MS + 1.5 mg l^{-1} BAP	0.43	1.27	1.76	2.23	2.90
Basal MS + 2.0 mg l^{-1} BAP	0.10	0.63	1.48	2.00	2.60
Basal MS + 3.0 mg l^{-1} BAP	0.21	0.62	1.25	1.72	2.30
Basal MS + 0.5 mg l^{-1} IBA	0.10	0.54	1.34	2.29	2.70
Basal MS + 1.0 mg l^{-1} IBA	0.30	0.80	1.64	2.23	2.80
Basal MS + 1.5 mg l^{-1} IBA	0.30	0.87	1.56	1.92	2.50
Basal MS + 2.0 mg l^{-1} IBA	0.20	0.82	1.69	2.03	2.40
Basal MS + 3.0 mg l^{-1} IBA	0.10	0.50	1.07	1.24	2.00
Basal MS + 0.5 mg l^{-1} GA_3	0.20	0.53	0.77	1.15	1.50
Basal MS + 1.0 mg l^{-1} GA_3	0.30	0.91	1.52	1.96	2.40
Basal MS + 1.5 mg l^{-1} GA_3	0.10	0.90	1.41	1.78	2.20
Basal MS + 2.0 mg l^{-1} GA_3	0.20	0.58	0.98	1.17	1.60
Basal MS + 3.0 mg l^{-1} GA_3	0.10	0.41	0.68	0.84	1.0
Basal MS (Control))	0.62	1.75	2.64	3.35	3.90
F-test	NS	**	**	**	**
SE.m±	-	0.23	0.29	0.27	0.41
CD at 5%	-	0.79	0.98	0.99	0.98

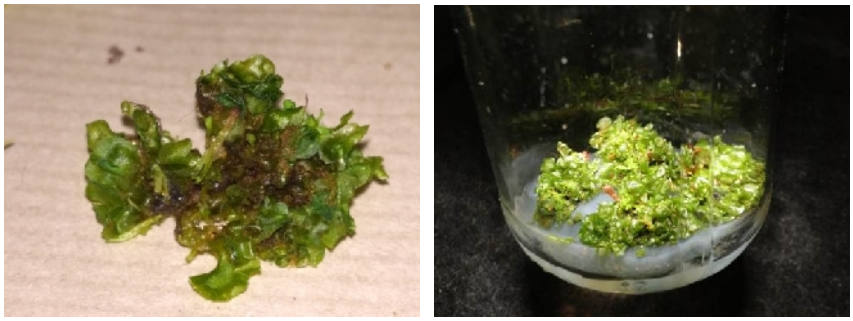

Fig. 1. Gametophyte development after sporeinoculation on basal media.

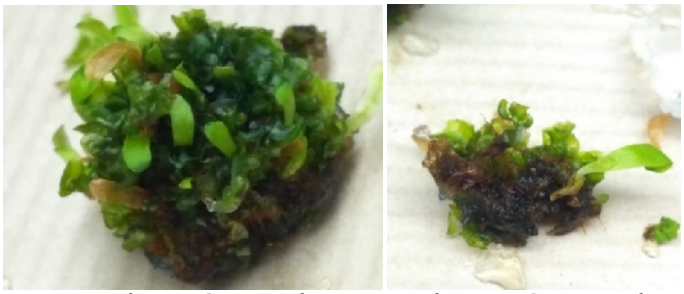

Fig. 2. Emergence of sporophyte (st) from centre of gametophyte (gt) after 60 days.

Further, data recorded up to 150 days showed (Fig. 3), the highest number of sporophytic transformation from gametophyte with media containing no growth regulators (2.64, 3.35 and 3.9). In respect of the number of the sporophytes emerged from gametophytes, in the initial days of observation made (45 and 60 days), the presence of BAP at lower amounts such as 1 mg l^{-1} resulted in significantly increased number of emergence of sporophyte. However, the observations made after 75 days of culture and thereafter up to 150 days, the number of sporophytes produced was significantly higher in the media devoid of any growth regulator. It appears from the results that initiation of sporophyte from the cultured gametophyte require a stimulus of BAP (1.0 mg l^{-1}).

Fig. 3. Fully developed single sporophyte.

In the later stages, from the observations made after 75 days, increased production of sporophyte from gametophyte in media with no growth regulator suggest that the gametophyte might have had the required cytokinin content produced endogenously with time. The results also suggest the continued presence of exogenously applied growth regulators in the media was not beneficial.

Similar findings of initial presence of BAP favouring production of sporophyte and continued presence of BAP for a longer duration in media as not beneficial was reported by Camloh *et al*., (1994); Camloh (2006) in their investigations on *Platycerium bifurcatum.*

Mean length of sporophyte after 150 days: Gametophytes were sub-cultured on MS media with different concentrations of growth regulators. After 150 days of culture, the mean length of sporophytes was observed. The mean length of sporophytes was found to be significantly higher (Fig. 3) in media containing GA_3 at 2 mg l^{-1} (3.56 cm) (Table 1.).

The presence of GA_3 in the media as one of the treatments favoured increase in the growth of the sporophyte produced. The length of sporophyte produced in media with 2 mg l^{-1} GA was significant than all other GA concentrations as well as media containing other growth regulators at various concentrations. The role of GA_3 in cell elongation is a well-established factor. Hence the present observation was recorded in our investigations. Similar results were reported by Taha *et al.,* (2011) in *Platycerium coronarium.*

CONCLUSION

From the study it has been found that Staghorn fern needs stimuli for the alternation of generation. In the natural process, it was found to be taking much time for transformation from one stage to another, while by external application of different growth regulators have led to a result in reducing the transformation time and better development of sporophytes. It is clearly evident that the gametophytes do respond to growth hormones. And there is a greater scope in future for developing protocol for sporophytes development in shorter time and hardening of plantlets, which is an important need of the hour.

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Conflict of Interest. Nil.

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