Reprint **ISSN 1997-2571 (Web Version)**

Journal of Innovation & Development Strategy (JIDS)

(J. Innov. Dev. Strategy)

Volume: 6 **Issue: 1** **April 2012**

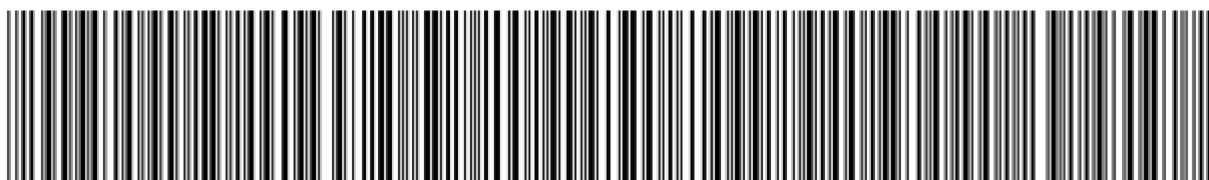
J. Innov. Dev. Strategy 6(1):81-85(April 2012)

TISSUE CULTURE PROPAGATION OF TROPICAL ORCHID *(Phaius tankervilliae)* PLANT

N. SULTANA, T.A. JAHAN, T.K. BARAI, M.S. AKHTER AND N. ARA

GGF
Nature is Power

An International Scientific Research Publisher
Green Global Foundation©
Publication and Bibliography Division
100 Leeward Glenway
Apartment # 1601
M3c2z1, Toronto, Canada
E-mails: publication@ggfagro.com, editor@ggfagro.com
http://ggfagro.com/ejournals/current issues



JIDS** issn 1997-2571, HQ:19-10 central place, saskatoon, saskatchewan, s7n 2s2, Canada

ISSN-1997-2571 (Online)
J. Innov. Dev. Strategy 6(1):81-85(April 2012)

TISSUE CULTURE PROPAGATION OF TROPICAL ORCHID *(Phaius tankervilliae)* PLANT

N. SULTANA[1*], T.A. JAHAN[2], T.K. BARAI[3], M.S. AKHTER[4] AND N. ARA[5]

[1] Department of Microbiology, Faculty of Biological Science and Technology , Jessore Science and Technology University, Jessore - 7408; [2]Department of Mathematics and Natural Sciences, BRAC University, 66 Mohakhali, C/A, Dhaka- 1212, Bangladesh; [3]Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh, Bangladesh; [4]Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna – 9208, Bangladesh; [5]Department of Pharmacy, University of Development Alternative, Dhanmondi, Dhaka, Bangladesh.

*Corresponding author & address: Nigarin Sultana, E-mail- nigarin4@yahoo.com
Accepted for publication on 25 March 2012

ABSTRACT

Sultana N, Jahan TA, Barai TK, Akhter MS, Ara N (2012) Tissue culture propagation of tropical orchid *(Phaius tankervilliae)* plant. *J. Innov. Dev. Strategy.* 6(1), 81-85.

Phaius tankervilliae is a famous ornamental plant which is rarely found in Bangladesh. Its beauty and uniqueness made it an important candidate for tissue culture studies especially for regeneration and multiplication purposes. Here we for the very first time report a rapid and reliable method for the regeneration of *Phaius tankervilliae in vitro*. Axillary buds explants from one year old *Phaius tankervilliae* plant were cultured on MS (Murashige and skoog's, 1962) medium without any hormone supplementation. Multiplication was carried out by using 1 mg/lit. and 0.1 mg/lit., BAP (6-benzylaminopurine) and NAA (α-naphthalene acetic acid), respectively. For elongation purpose, 0.5 μM GA3 (gibberellic acid) was used. 0.1 mg/lit. NAA was used to stimulate extensive root development within 1 month. After successful regeneration, the plantlets were acclimatized into the soil. This study is undertaken to perform in vitro clonal propagation and acclimatization of *Phaius tankervilliae* in order to get hundreds of *Phaius tankervilliae* plants in the laboratory in comparatively less time. Hence, it would be possible to regenerate *Phaius tankervilliae* plants in the laboratory and after successful acclimatization.

Key words: *Phaius tankervilliae, axillary Buds, micropropagation, murashige and skoog, acclimatization*

INTRODUCTION

Phaius is a genus of large, mostly terrestrial plants; it is composed of some twenty species that produce tall spikes of showy flowers. *Phaius* species inhabit tropical Asia, into China, Japan, Australia, and west to Africa and Madagascar. The most commonly cultivated is *P. tankervilliae* (syn. *grandifolius*), which has been in continual western cultivation since 1778, when the first plants were imported from China. *P. tankervilliae* is a deciduous, terrestrial orchid, which is more commonly known as the "Nun's Orchid" or the "Veiled Orchid (Berry 1982). Although proper research could not be made on the *Phaius tankervilliae*. Medicinal properties but in South America it is a routine strategy to use the crushed petals of *Phaius tankervilliae* and the juice from its berries to treat skin ailments, freckles and small blisters and rashes. The flower of *Fuchsia arborescens* are even eaten and being used on bites, scratches and grazes and its juice has a relieve effect on itching and taking away the redness. They are also used to relieve inflamed blisters and sunburn. Fuchsia flowers and berries are used to make a superb jelly that include lemon juice, apple juice and a dash of brandy which is used as a remedy for sore throat, tonsillitis and to strengthen the voice (Roberts 2000). In vitro regeneration of plantlets from stem nodal explants has been reported as an efficient and rapid strategy for large scale propagation of certain important plants like *Mucuna pruriens* (Faisal *et al.* 2006; Sathyanarayana *et al.* 2008), *Jatropha Curcas* L. (Shrivastava and Banergee, 2008), *Stevia rebaudiana* Bertoni (Ahmed *et al.* 2007), *Azadirachta indica A. juss* (Chaturvedi *et al.* 2004) and an aromatic medicinal herb, *Ocimum Basilicum* L. (Sahoo *et al*. 1997). The callus proliferation and regeneration of *Fuchsia hybrida* was successfully carried out by Chow *et al*. in 1990, which was reported to occur more rapidly than that which was observed in callus derived from ovary tissues (Dabin and Vaerman, 1985). Rapid development of Axillary buds from shoot-tips and nodes of 18 cultivars of *Fuchsia hybrida* has been obtained on solid Murashige and Skoog medium with BAP (6- benzylaminopurine) and an auxin (Kevers *et al.* 2003). Tissue culture and rapid propagation of *Fuchsia alba-coccinea* Hort was also carried out by using explants of stem fragments with nodes (Fugen *et al.* 2006). We selected *Phaius tankervilliae* for tissue culture studies. We performed in vitro initiation, multiplication, elongation, rooting and acclimatization of *Phaius tankervilliae*.

MATERIALS AND METHODS

The experiment was conducted from June 01 to December 01, 2010 at Plant Tissue Culture laboratory of the Department of Genetic Engineering and Biotechnology, Jesssore Science and Technology University, Jessore, Bangladesh to clarify the effective concentration of growth regulators for *in vitro* seed germination and plantlets development of orchid plant.

Culture and Condition

Nodal segments were cultured on ¾ Murashige and Skoog (Murashige and Skoog, 1962) media supplement for the initiation of *Phaius tankervilliae* with 3% sucrose and 0.8% agar. After adding the hormones, the pH of medium was adjusted to 5.75. The culture containing media were autoclaved at 121°C for 20 min. All the cultures were incubated in a culture chamber maintained at a temperature of 23-25°C under 16-h photoperiod provided by cool white fluorescent tubes.

Copyright© 2012 Green Global Foundation

Table. 1 Composition and preparation (Macronutrients, Micronutrients, Vitamins and other supplements) of the modified Murashige and Skoog (MS) medium

Constituent	MS Medium [mg l^{-1}]	Concentrated Stock Solution [mg l^{-1}]	Working Solution [mg l^{-1}]	Storage [°C]
Macronutrients				
Ammonium nitrate	1650	33000	50	4
Calcium chloride dihydrate	440	8800	50	4
Magnesium sulphate heptahydrate	370	7400	50	4
Potassium nitrate	1900	38000	50	4
Potassium phosphate monobasic	170	3400	50	4
Micronutrients (Trace elements)				
Boric acid	6.2	620	10	-18
Cobalt chloride hexahydrate	0.025	25	1	-18
Cupric sulphate pentahydrate	0.025	25	1	-18
Manganese sulphate monohydrate	16.9	338	50	-18
Molybdic acid sodium dihydrate	0.25	250	1	-18
Potassium iodide	0.83	830	1	-18
Zinc sulphate heptahydrate	8.6	172	50	4
Sodium iron (III) ethylene diamine tetraacetic acid	37.5	750	50	4
Organics supplement (Vitamins)				
Glycin	2	40	50	4
Myo-inositol	100	2000	50	4
Nicotinic acid (B3)	0.5	50	10	-18
Pyridoxine hydrochloride (B6)	0.411	41.1	10	-18
Thiamine hydrochloride (B1)	0.1	10	10	-18
Buffer				
Sodium dihydrogen phosphate dihydrate	192.5	3850	10	4
Other Supplements				
Sucrose		30000		
Phyto-Agar		6000		
Gelrite		2500		

Preparation of Phaius tankervilliae explant

The healthy plant, having 8-10 leaves and stem length of 7-11 cm were selected as the source of explants. The plants were systematically washed with running tap water for at least 1 h. plants were dried out and the lowest part of the stem bearing roots was detached. All the mature leaves present at the nodes were detached with a sterilized pointed blade in such a way that at least ½ -1 in of the leaf base remained intact with the stem node explants, after incomplete removal of the leaves. A slanting cut was given at the base of the leaf. The cut was given very carefully in such a way that the leave sheath remained intact and enclosed whole of the nodal region under it.

Surface sterilization and explants sizing

All the pre explants were thoroughly washed with sterilized distilled water followed by a dip in solution of 20% bleach and few drops of tween20 (polyoxyethylene (20) sorbitan monolaurate) for 15 minutes with constant shaking. After this treatment, the pre-explants were given 4–5 thorough washings with autoclaved double distilled water to remove any trace of the Bleach and few drops of tween 20. Stem nodal segments of 1.5 cm in size were cut under sterile conditions as per the steps mentioned above. All the explants were cultured in the Murashige and Skoog media. The cultures were incubated at 25°C under 16-h photoperiod provided by cool white fluorescent tubes. The cultures were observed periodically.

Iniciation Media

Nodal segments were cultured on ¾ Murashige and Skoog (1962) without addition of any growth regulators.

Multiplication Media

After successful initiation the single shoot were transferred in ¾ Murashige and Skoog (1962) with the addition of NAA (α-naphthalene acetic acid) and BAP (6- benzylaminopurine). The concentrations of BAP and NAA used were identical to those reported by Kevers *et al.* in 2003 i.e. 1 mg l−1 and 0.1 mg l−1, respectively.

Shoot Elongation Media

After successful multiplication the micro shoot were transferred in ¾ Murashige and Skoog (1962) media (pH=5.75) with the addition of hormone GA3 (gibberellic acid). The concentration of GA3 was set to be 0.5 µM.

Rooting Media

The isolated shoots (3-4cm) with three to four pairs of leaves were transferred to ¾ MS medium supplement with the addition of hormone NAA. (α-naphthalene acetic acid) 0.1 mg / liter NAA solution was used to stimulate rooting.

Acclimatization

Plantlets with well-developed shoots and roots were removed from the culture medium, washed gently under running tap water and transferred to plastic pots containing sterile garden soil, peat moss under diffuse light conditions. Potted plantlets were covered with a transparent polythene bag to ensure high humidity and watered every 3 days with ¾ MS salt solution for 2 weeks. Polythene bag were opened after 3 weeks in sort to acclimatize plants to field conditions. After 5 weeks, acclimatized plants were transferred to pots containing standard soil and maintained in a greenhouse under normal day length conditions.

RESULTS AND DISCUSSION

Initiation of *Phaius tankervilliae*

We cut the shoot in such a way that each piece of 1.5cm long shoot contained two axillary buds and cultured them into the initiation media. We used ½, ¾ and standard MS media to check optimal response. After 3-4 days, most prominent induction of leaves was observed in ¾ MS. Leaves form each bud were emerged and continues to grow during first week. After two weeks, one prominent shoot of 4 cm was emerged from each bud having a number of leaves. At the end of 3rd week, the shoot length increased up to 4.5 cm. The explants were kept in the same media for three weeks until the shoot reaches a length of 4cm. As initiation media is ¾ MS without hormone, it means that initiation of *Phaius tankervilliae* does not require any hormone. Whereas, the control plant was set by planting a 10 cm long leafless shoot of *Phaius tankervilliae* in the soil. The induction of leaves could not be observed before twenty days. It suggests that ¾ MS media has enough potential to stimulate rapid initiation in *Phaius tankervilliae.*

Fig. 1. Showing *in vitro* root formation in *Dendrobium* orchid on different concentrations of IBA at 30 DAI multiplication of *Phaius tankervilliae.*

After successful initiation of *Phaius tankervilliae*, we divided the initiated shoot in such a way that each piece should not be more than 1 cm and must contain two nodes. Then the pieces were cultured in sterilized ½, ¾ and standard MS media in the presence of 1 mg l−1 and 0.1 mg l−1, BAP (6-benzylaminopurine) and NAA (α-naphthalene acetic acid), respectively. These concentrations of BAP and NAA used here were same as used by Kevers and colleagues (Kevers *et al.* 2003). We observed more extensive multiplication in ¾ MS media as compare to other concentrations of MS media used.

After one week, three shoots were emerged from each node. Shoots became 1.5 cm long at the end of third week. But it is worth to mention that the shooting was extensive in all the explants suggesting the suitability of the ¾ MS medium in combination with two hormones i.e. BAP and NAA. BAP or Cytokinin helps in rapid induction of multiplication of shoots, buds, or meristems (Rajore *et al.* 2002). Advantage of BAP over Kn for multiple shoot induction was established in *Pterocarpus marsupium* (Suresh and Ajay, 2004), *Sapium sebiferum*, (Siril and Dhar, 1997). Sathyanarayana *et al.* (2008) observed steady increase in number of shoots while using BAP up to 3.55 M. Cytokinin concentrations beyond these adversely affected the shoot growth, as the regenerated shoots became small and thick. The small nature of shoot formation parallel to increased concentration of BAP in the medium was also reported in *Eupatorium* (Martin 2004) and *Orthosiphon* (Lai-Keng and Leng, 2004) For Fuchsia magellanica Lam. we observed that extensive multiplication was achieved at 0.225 M BAP. NAA in combination with BAP helps in stem elongation. It has been recognized that the addition of NAA promotes the proliferation and elongation of shoots in *Petasites hybridus* (Wldi *et al.* 1998), *Mucana pruriens* (Faisal *et al.* 2006), E*ucalyptus grandis* (Luis *et al*. 1999) and *Hybanthus enneaspermus* (Prakash *et al.*

1999). BAP and NAA are effectual for multiplication purpose in tissue culture studies, as described by Kevers and colleagues (Kevers *et al.* 2003).

Table 2. Effect of BAP concentrations on shoot multiplication and shoot length in *Phaius tankervilliae.* Values represent means ± SE of three independent experiments with ten replicates each

BAP [mg l^{-1}]	Number of shoots per explant	Shoot length [cm]
0.5	7.3 ± 0.8	4.5 ± 0.5
1.0	9.8 ± 0.8	4.2 ± 0.7
1.5	16.8 ± 1.6	2.3 ± 0.4

Our results also showed that in contrast to the control (¾ MS media without hormone supplementation) which shows no induction of shoot multiplication, good multiplication *Phaius tankervilliae* was achieved by using a combination of these two hormones in ¾ MS media. This strongly suggests the efficiency of BAP and NAA in promoting extensive lateral branching.

Shoot Elongation of *Phaius tankervilliae*

Elongation of shoots was found to be most excellent on GA3 containing medium over other media supplemented with different hormones. Transferring the micro shoots to MS + GA3 (2.89 - 14.43 _*M*) produced better elongation (data not shown), with GA3 (2.89 _*M*) producing maximum elongation of 4.0 cm (Sathyanarayana *et al.* 2008). The gibberellins are concerned in several physiological process regulations such as seed germination, initiation and growth of flowers and shoot elongation (Rkhis *et al.* 2006 and Ben Nissan *et al.* 2004) observed that GA3 (gibberellic acid) has role in cell elongation in stems. At this time for shoot elongation purpose, 0.5 cm long shoots were cut and transferred to ½, ¾ and standard MS media supplemented with GA3 (0.5gm liter). In ¾ MS media, shoots were elongated up to 4 cm within eight days while in the two other concentrations of MS media (1/2 , ¾ MS), no prominent response can be achieved. There was shown to be gradual but continuous increase in the length of shoots. The elongation was shown to be solely depends on the presence of GA3 hormone because no significant shoot elongation was observed in the control jars containing ¾ MS media without GA3 supplementation.

Root development of *Phaius tankervilliae*

In vitro rooting of regenerated shoots was achieved on ¾ MS medium equipped with 0.1mg l-1NAA (α-naphthalene acetic acid), though the other concentrations (1/2 and standard MS media) were also tested. NAA as a key hormone in inducing roots are also reported in several established micro-propagation protocols like *M. pruriens* var. (Chattopadhyay *et al.* 1995), *Jatropha curcas* (Rajore *et al.* 2002), *Hyptis suaveolens* (Britto *et al.* 2001), *Pisonia alba* (Jagadish *et al.* 1999) and *M. pruriens* var. Utilis (Sathyanarayana *et al.* 2008). We observed extensive root development in the presence of NAA within one month. Whereas, in control plant no NAA was added, root development was comparatively negligible. It proved the potential of NAA in root development of *Phaius tankervilliae.*

Acclimatization of *Phaius tankervilliae*

After successful in vitro rooting in *Phaius tankervilliae*, we performed acclimatization i.e. planting the *in vitro* regenerated *P. tankervilliae* plants into the pots containing normal soil. This study showed that in vitro clonal propagation of *Phaius tankervilliae* can be more rapidly achieved i.e. within 3 months as compare to the control which showed only induction of leaves within the same period of time.

CONCLUSION

We have performed in vitro clonal propagation of *Phaius tankervilliae* and found that it is easier to grow and propagate *Phaius tankervilliae* in Tissue culture lab compare to Botanical garden (Center for plant conservation, Herbarium) as compare to the soil (control). Here, we also conclude that ¾ MS media is well suited as compare to ½ and standard MS media for the in vitro regeneration of *Phaius tankervilliae* from stem nodules explant. Additionally, for multiplication, elongation and root development, a combination of NAA (α-naphthaleneacetic acid) and BAP (6-benzylaminopurine), GA3 (gibberellic acid) and NAA alone are required to be added into the ¾ MS media, respectively. Now, it is possible to grow *Phaius tankervilliae* rapidly in vitro conditions with minimum hormonal supplementation.

REFERENCES

Ahmed MB, Salahin M, Karim R (2007) An efficient method for clonal propagation of a newly introduced sweetener plant (*Stevia rebaudiana* Bertoni) in Bangladesh. *Am Eurasian J Sci Res*; 2(2), 121-125.

Ben-Nissan G, Lee JY, Borohov A, Weiss D (2004) GIP, *Petunia hybryda* GA –Induced cysteine – rich protein: A possible role in shoot elongation and transition to flowering. *The Plant J*; 37(2), 229-238.

Berry PE (1982) The systematics and evolution of *Fuchsia* sect. *Fuchsia* (Onagraceae). Annals of the Missouri Botanical Garden 69, 1–198.

Britto SJ, Robinson JJ, Natarajan E, Arockiasamy DI (2001) Micropropagation of *Hyptis suaveolens* (L.) Poit through nodal culture. Adv. Plant Sci.; 14(11), 561-565.

Chattopadhyay S, Datta SK, Mahato SB (1995) Rapid micropropa- gation for *Mucuna pruriens* f. *pruriens* L. Plant Cell Rep; 15, 271-273.

Chaturvedi R, Razdan MK, Bhojwani SS (2004) *In vitro* clonal propagation of an adult tree of neem (*Azadirachta indica* A. Juss) by forced Axillary branching. Plant Sci; 166, 501-506.

Chow YN, Harvey BMR, Selby C (1990) An improved method for callus proliferation and regeneration of *Fuchsia hybrida*. Plant Cell, Tissue and Organ Culture; 22, 17-20.

Dabin P, Vaerman AM (1985) Callogenese et organogenese chez deux cultivars de fuchsia (Constance et Swingtime). Bull Soc R Bot Belg; 118, 172-178.

Faisal M, Siddique I, Anis M (2006) *In vitro* rapid regeneration of plantlets from nodule explants of *Mucuna prureins*-a valuable medicinal plant. Ann Appl Biol 2006; 148, 1-6.

Fugen G, Ruiqing C, Zhigang W, Bingyao S (2006) Tissue culture and rapid propagation of *Fuchsia alba-coccinea* Hort. Zhiwu Ziyuan Yu Huanjing Xuebao; 15(3), 55-59.

Jagadish CKS, Rachappaji S, Gowda KRD, Thara SKJ (1999) *In vitro* propagation of *Pisonia alba* (Linn.) Sapanogae (Lettuce Tree), A threatened species. Phytomorpholog; 49, 43-47.

Kevers CL, Coumans-Gilles MF, Coumans M, Gaspar TH (2003) *In-vitro* vegetative multiplication of *Fuchsia hybrida*. Sci Hortic; 21(1), 67-71.

Lai-Keng C, Leng W (2004) Plant regeneration from stem nodal segments of *Orthosiphon stamineus* Benth, a medicinal plant with diuretic activity. *In Vitro* Cell Dev Biol Plant; 40, 115-118.

Luis PBC, Adraine CMGM, Silvica BRCC, Anna CM (1999) Plant regeneration from seedling explants of *Eucalyptus grandis – eucalyptus urophylla*. Plant Cell Tissue Organ Cult., 56, 17-23.

Martin KP (2004) Rapid axillary bud proliferation and *ex vitro* rooting of *Eupatorium triplinerve*. Biol Plant; 47, 589-591.

Murashige T, Skoog FA (1962) Revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol Plant; 15, 473-497.

Prakash E, Sha VKPS, Sairam RP, Rao KR (1999) Regeneration of plants from seed derived callus of *Hybathus enneaspermus* L. Muell, a rare ethanobotanical herb. Plant Cell Reports 1999; 18, 873-878.

Rajore S, Sardana J, Batra A (2002) *In vitro* cloning of *Jatropha curcus* (L.) *J Plant Biol*.; 29(2), 195-198.

Rkhis AC, Maalej M, Messaoud SO, Drira N (2006) *In vitro* vegetative growth and flowering of olive tree in response to GA3 treatment. *Afr J Biotechnol*; 5(22), 2097- 2302.

Roberts M (2000) Edible and medicinal flowers. Spearhed, 20.

Sahoo Y, Pattnaik SK, Chand PK (1997) *In vitro* clonal propagation of an aromatic medicinal herb *Ocimum Bacilicum* L. (sweet Basil) by Axillary shoot proliferation. In Vitro Cell Dev Biol Plant 1997; 33, 293-296.

Sathyanarayana N, Kumar TNB, Vikas PB, Rajesha R (2008) *In vitro* clonal propagation of Mucuna prureins varutilis and its evolution of genetic stability through RAPD markers. *Afr J Biotechnol*; 7(8), 973-980.

Shrivastava S, Banergee M (2008) *In vitro* clonal propagation of physic nut (*Jatropha Curcas* L.): Influence of additives. IJIB; 3(1), 73-79.

Siril EA, Dhar U (1997) Micropropagation of mature Chinese tallow tree (*Sapium sebiferum* Roxb.). Plant Cell Rep. 16, 637-640.

Suresh C, Ajay KS (2004) *In vitro* shoot regeneration from cotyledonary node explants of a multipurpose leguminous tree, *Pterocarpus marsupium* Roxb. *In vitro* Cell Dev Biol Plant 2004; 40, 167-170.

The Encyclopædia Britannica: A Dictionary of Arts, Sciences, Literature and General Information, 11th Edition: *Volume XI, Franciscans to Gibbons* The Encyclopedia Brittanica Company: New York, pp. 272.

Wldi E, Schaffner W, Berger KB (1998) In vitro propagation of *Petasites hybridus* (Asteraceae) from leaf and petiol explant and from inflorescence buds. Plant Cell Rep; 18, 336-340.