Reprint **ISSN 1991-3036 (Web Version)**

International Journal of Sustainable Crop Production (IJSCP)

(*Int. J. Sustain. Crop Prod.*)

Volume: 6 **Issue: 2** **August 2011**

***Int. J. Sustain. Crop Prod.* 6(2): 1-5 (August 2011)**

IN VITRO SEEDS GERMINATION AND PLANTLETS DEVELOPMENT OF *SILVIUS APPENDICULATUS* (Orchidaceae)

M.N. HASAN, A. ANSARI, M.S.Z. SHAHRIAR, M.N.K. AZAM, S.M.A. AWAL

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

IJSCP** issn 1991-3036, HQ:19-10 cantral place, saskatoon, saskatchewan, s7n 2s2, Canada

ISSN-1991-3036 (Online)
Int. J. Sustain. Crop Prod. 6(2):1-5(August 2011)

IN VITRO SEEDS GERMINATION AND PLANTLETS DEVELOPMENT OF *SILVIUS APPENDICULATUS* (Orchidaceae)

M.N. HASAN[1], A. ANSARI[2], M.S.Z. SHAHRIAR[3], M.N.K. AZAM[4], S.M.A. AWAL[5]

[1,2]Lecturer, Department of Genetic Engineering and Biotechnology, Jessore Science and Technology University, Jessore, Bangladesh; [3]Social welfare officer, Dhaka, Bangladesh; [4]Biotechnology and Genetic Engineering Department, University of Development Alternative, Dhanmondi, Dhaka, Bangladesh; [5]Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna – 9208, Bangladesh.

Corresponding author & address: Md. Nazmul Hasan, E-mail: nazmul_bt19@yahoo.com
Accepted for publication on 6 August 2011

ABSTRACT

Hasan MN, Ansari A, Shahriar MSZ, Azam MNK, Awal SMA (2011) *In vitro* seeds germination and plantlets development of *Silvius appendiculatus* (Orchidaceae). *Int. J. Sustain. Crop Prod.* 6(2), 1-5.

Bangladesh is rich in orchids, with 159 species and two varieties under 63 genera (Huda *et al.*1999). These species are distributed mainly in the hilly areas of greater Sylhet, Chittagong and Mymensingh districts. Seeds of *S. appendiculatus* were germinated successfully and grew well in the *in vitro* culture. The best medium were observed for germinating the *S. appendiculatus* seeds, *viz*. Knudson-C (KnC) basal medium supplemented with 30% (v/v) maturity coconut water/milk (CW). All the seeds were observed those overall germination, protocorms formation and seedling development (about 100%). The best medium for growing and development of seedlings to become fully expanded plantlets was determined half strength of Murashige and Skoog (1/2MS) medium supplemented with 40% (v/v) CW. The plantlets of *S. appendiculatus* were resulted from these research approximately more than 2000 individuals. The fully expanded seedlings were transferred to perforated plastic pots and grow in the screen house.

Key words: *Orchidaceae, Silvius appendiculatus, in vitro, seed germination, coconut water*

INTRODUCTION

Orchidaceae were known as ornamental plants which have attractive flowers and high purchase prices. Around 35000 species under 800 genera of orchidaceae grow in the world (Singh *et al.* 2007). The other scientist reported that family of Orchidaceae lies between 25000 and 30000 different species (Falsetto 2008). The Royal Botanical Gardens of Kew listed 880 genera and accepted nearly 22,000 species. The exact numbers of Orchidaceae species is unknown because of taxonomic disputes. One of attractive flower of Bangladeshi's orchids is *Silvius appendiculatus.* The *S. appendiculatus* belongs long lasting big flower. The appearance of *S. appendiculatus* and their flower were shown in Figure 1.

Wide areas of orchid habitat in the forest were destroyed for utilization as properties development, mining industry, and plantation companies. Therefore, we need to conserve and propagate the attractive orchids flower for keeping the sustainability of orchid germplasm. Propagations of orchids through asymbiotic seeds germination were reported at *Epidendrum ibaguense* Kunth (Hossain 2008), *Geodorum densiflorum* (Lam.) Schltr. (Bhadra and Hossain, 2003), *Oncidium* sp. (Kalimuthu *et al.* 2007), and *Aplectrum hyemale* (Muhl. Ex Willd.) Torr. (Lauzer *et al.* 2007). The propagation methods were initiated since 1917 by Dr. Lewis Knudson which discovered the composition of the essential nutrients for germination orchid seeds (Arditti and Ghani, 2000). The attractive flowers of orchidaceae over the world were germinated by using different composition of basal medium and modification of Knudson's methods.

The *in vitro* seeds germination and micropropagation were known as effective methods for resulting mass seedling of orchid. The orchids produce abundant seeds in their fruit pods. In the previous studies were reported that orchid produced 1,300 to 4,000,000 seeds (Pierik 1987). Large seeds of orchidaceae will germinate in the *in vitro* condition if we culture in the appropriate germination medium. Propagation of one pod of orchidaceae will result a large amount of plants if the appropriate method is followed. Several scientists discovered appropriate medium for propagation by certain species of orchidaceae, such as *in vitro* seeds germination and seedlings development of *G. densiflorum* were achieved by using MS basal medium supplemented with various growth regulators (Bhadra and Hossain, 2003), *E. ibaguense* were declared success by using modification of MS, Phytamax, Mitra *et al.* and Knudson C (KnC) basal medium (Hossain, 2008), *Oncidium* sp. was achieved by using MS supplemented with 2 mg/l BAP (Kalimuthu *et al.* 2007), and *Rhynchostylis gigantean* (Lindl.) Ridl. was found successful by using half-strength MS medium supplemented with growth regulators (Li and Xu, 2009). Therefore, for achieving a successful propagation of the certain species of orchidaceae need to be modified and enriched the basal medium used. This study was proposed to achieve the appropriate medium for asymbiotic germination and seedling development of *S. appendiculatus.*

MATERIALS AND METHODS

The experiment was conducted from February 01 to September 01, 2011 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.

Preparation of explants

The green pods of *S. appendiculatus* were harvested from Chittagong district of Bangladesh for using as plant materials and propagated through *in vitro* seed germination. The dry petals attached the pods were removed,

dipped the pods quickly into 95% alcohol and flame over a spirit lamp then transferred into a sterile Petri dish. The pods were cut across into 2 halves by using surgical blade. Before inoculating the seeds, the sizes of the seeds were measured by using a binocular microscope.

Seeds germination

The seeds were spreads on the surface of KnC solidified medium supplemented with various concentration of mature coconut water (CW), *viz*. 0, 10, 20, 30, and 40% as treatments. The pH of all the media were adjusted to 5.2 before pour into 200 ml glass vessels. The glass vessels were filled in 25 ml medium then sterilized of the medium with 120^{o}C temperature and 1.2 cm^{2}/kg pressures for 25 minutes by using autoclave apparatus. After the seeds inoculation on the surface of solidified medium, the culture vessels were incubated in the culture room with 25^{o}C temperature and 12 h photoperiod illumination by using tube lamp. The effect of treatments toward seeds germination was weekly recorded. The duration of seeds germination process was terminated when the protocorm-like bodies was formed.

Seedling development

The basal mediums used for seedling development were KnC and 1/2MS. Both KnC and 1/2MS mediums were supplemented with 0, 10, 20, 30, and 40% CW as treatments. The protocorms with their shoot primordial were inoculated on the surface of both KnC and ½ MS medium. The culture vessels were maintained in the same culture room of the seeds germination point. The growth responses of seedling were also weakly observed. The experiments were terminated when the seedlings were fully developed (belonging roots, 3-4 leaves, and 4-5 cm high) and ready for acclimatization.

RESULTS AND DISCUSSION

Characteristic of seeds

Orchid propagation by using seeds is very important point for achieving plenty of plants. Seed germination of orchidaceae could be done through asymbiotic and symbiotic methods. The asymbiotic methods were used for examining of seeds germination of *S. appendiculatus*. Several authors mentioned that the seeds of orchidaceae differ from the seeds of many other plants, they were known as lack or no endosperm in their seeds (Pierik 1987; Kalimuthu *et al*. 2007). The seeds from green pods of *S. appendiculatus* were used as explants that have yellowish color, tapering shape, 40.2 µm longs, 11 µm wide, and no endosperm incorporated with the embryo (Figure. 2). Seeds of *Calanthe discolor* are 30 µm long (Miyoshi and Mii, 1995). The seed sizes of orcidaceae might depend on the species. Generally, orchid seeds were 1.0–2.0 mm long and 0.5-1.0 mm wide, produced large number (1,300-4,000,000) of seeds: per pod (Pierik 1987). Therefore, a large potential of seedlings will achieve from one pod of certain species of orchidaceae if germination methods were discovered.

Seeds germination

The seeds of *S. appendiculatus* were cultured on KnC medium supplemented with various concentration of CW. The responses of the seeds toward the media were observed at three development stages, namely color change of the seeds, swollen seeds, globular shape, and protocorm-like bodies (Table 1). Batygina *et al.* (2003) reported that the stages of seeds germination are swollen seeds, testa breaking, protocorm before organ differentiation, and protocorm with leaf premordium. The seeds color was changed in one week after inoculation to the culture medium. Based on RHS color charge, the color of seeds used as explants is light greenish yellow (8B) and then change to become strong oranges yellow (163B) in seven days after culture. Seeds color change in culture vessels might predict that the seeds have been absorbed water and nutrient as inducer of embryo cell proliferation and testa breaking, then globular formation. The globular and protocorm structures were formed in 28 days and 35 days after culture respectively. The best medium was achieved for development globular and protocorm structures in *S. appendiculatus*, *viz*. KnC basal medium supplemented with 30% CW (Table 1). The performance of globular and protocorm formation was shown in Figure 3B and 3C respectively. In general, coconut water (CW) contained inorganic ion, amino acid, enzymes, vitamin, growth regulator and organic acid that might influences of plant cell growth. The CW contains: K, Na, Ca, Mg, Fe, Cu, P, and S, vitamin C, nikotinat acid, pantotenat acid, folat acid, biotin, and riboflavin (Yong *et al.* 2009). The CW was reported as inducer of sel proliferation and differentiation of several species of orchidaceae, such as enhanced protocorm proliferation of *Cymbidium* (Kusumoto 1980), induced high percentage of protocorm formation of *Ophrys* species (Kitsaki *et al.* 2004), and high frequency regeneration of *Dendrobium malones* by addition CW and other organik compound in the medium (Anjum *et al.* 2006). The CW also enhancement proliferation and differentiation of non orchidaceae, such as callus and shoots of *Spinach oleracea* L. (Khayri *et al.* 1992), callus and roots growth of *Cucumis melo* var. Honey Dew.(Syafii *et al.* 2000).

The seeds were cultured on KnC medium withouth supplemented CW showed that no globular structures and protocorm were formed (Table 1). This indicated that the CW is very impotant supplement for seeds germination of *S. appendiculatus*. In the contrary, no seed germination was observed on the KnC medium with 0 and 10% CW. The seeds might be in dormant condition. When the seeds were transferred to the KnC medium

with 30% CW, they grew to form globular structure and then protocorm structure. These features indicated that the appropriate CW concentration was very important for germination of *S. appendiculatus* seeds.

Seedlings development

The protocorms with primordial shoot were transferred to both KnC and 1/2MS mediums supplemented with various CW concentrations. Growth responses of seedlings by KnC and 1/2MS medium were observed slightly different in plantlets. The 1/2MS medium induced more greenish color and produced higher number of plantlets than KnC medium (Tabel 2). Seed germination of *Epidendrum ibaguense* Kunth showed differences between MS and KnC medium (Hossain 2008). Li and Xu (2009) found that MS medium induced elongation of leaves and roots growth of *Rhynchostylis gigantean*. The composition of MS medium contained more nutrients than KnC medium. Therefore, the differences of plantlet performance might be caused indirect effect by certain nutrient, such as vitamin and microelements in the MS medium. Shadang *et al*. (2007) reported that MS medium is the best for further protocorm development.

The appropriate mediums for seedling development to become fully expanded plantlets were observed by both KnC and 1/2MS supplemented with 30 to 40% CW. The developments of seedlings were divided into three stages, *viz*. shoots elongation, shoots and leaves expanding, and roots elongation. It was observed that these three stages were developed within 7, 14, and 35 days respectively after the protocorm with shoot primordial transferred to the fresh mediums (Table 2). The appropriate medium for supporting fully expanded plantlets was 1/2MS and KnC medium supplemented with 40% CW. The appearance of seeds germination and seedlings development of *G. scriptum* was shown in Figure 4 and the performance of fully expanded plantlets were shown in Figure 4F. Several authors observed that the coconut water enhanced seeds germination and seedlings growth of Orchidaceae and other plants (Kusumoto 1980; Kitsaki *et al.* 2004; Puchooa 2004; Anjum *et al.* 2006). Piexe *et al.* (2007) investigated that CW and BAP successfully replaced zeatin in *Olea europaea* L. micropropagation. Another advantage of addition CW into basal medium was to induce large size seedling of *Vanda* hybrid (Mathews and Rao, 2003) and plantlets development of *Coelogyne ovalis* Lindl. and *Coelogyna nitida* (Wall.ex Don) Lindl. (Nongrum *et al.* 2007). Therefore, the protocol developed in this effort is acceptable for conservation and mass propagation of *S. appendiculatus*. Fully expanded plantlets with a strong root system were transferred from the culture vessel to the pots containing fern and maintained into the screen house (20% light). The seedlings were successfully established in the potting medium (Figure 5). The plants growth and development will be observed further.

Table 1. Growth of seeds in solidify KnC medium supplemented with various concentrations of Mature Coconut Water

Concentrations of MCW (%)	Color seeds change (in 7 days)		Swollen seeds (in 14 days)	Globular formation (in 28 days)		Protocorm formation (in 35 days)	
	Current	Change	Quantity	Quantity	Color	Quantity	Color
0	8B	163B	-	-	-	-	-
10	8B	163B	-	-	-	-	-
20	8B	163B	+	+	154C	+	154C
30	8B	163B	+++	+++	154A	+++	154A

Notes: RHS color chart: light greenish yellow (8B), strong oranges yellow (163B), vivid yellowish green (154A), brilliant yellowish green (154C), no Swollen seed, globular, and protocorm formation (-), small number (+), large numbers (+++).

Table 2. Seedling development by using solidified KnC and 1/2MS mediums supplemented with various concentrations of Mature Coconut Water

Conc. of MCW (% v/v)	Shoot initiation (in 7 days)	Shoots & leaves (in 14 days)		Roots (in 35 days)	Plantlets (in 35 days)	
		Shoots	Leaves		Height (cm)	Color
KnC						
0	+	-	-	-	-	-
10	+	+	+	-	-	150C
20	++	++	++	+	2.4	150B
30	+++	+++	+++	++	3.2	150B
40	++++	++++	++++	+++	4.0	150A
1/2MS						
0	+	+	-	-	-	149C
10	+	+	+	-	-	149C
20	++	++	++	+	2.8	149B
30	+++	+++	+++	+++	4.2	149B
40	++++	++++	++++	++++	5.5	149A

Notes: RHS color chart: brilliant yellowish green (149A-C; 150A-C), no growth (-), small numbers (+), moderate numbers (++), large numbers (+++), very large numbers (++++)

Figure 1. *S. appendiculatus* used as explants in this experiment. Vegetative features (A) and flower appearance (B)

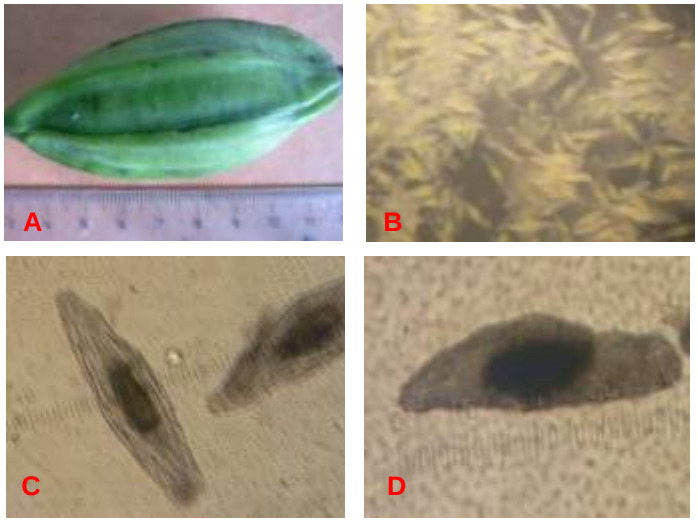

Figure 2. The pod and the seed of *S. appendiculatus* used as explants. Microscope enlargement 40x (B) and 100x (C and D), Seeds derived out the pod (B), wide size of seed (C), long size of seed (C)

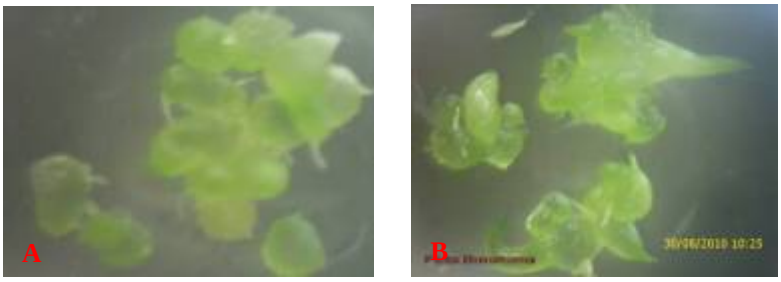

Figure 3. Appearance of globular and protocorm formation with microscope enlargement 40x. globular stages (A), and protocorm stages (B)

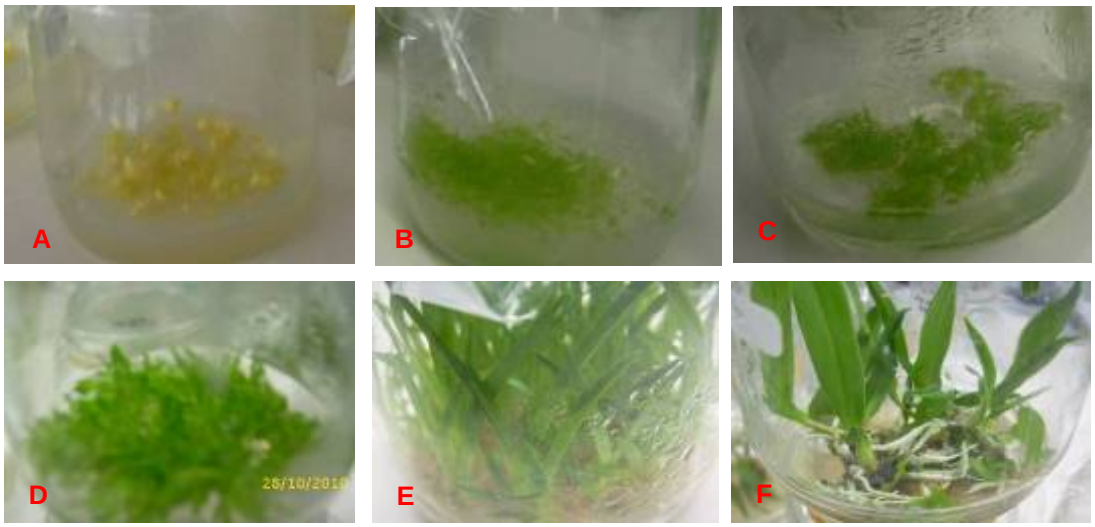

Figure 4. Growth and development of seeds to plantlets formation. Seeds inoculation (A), globular stage (B), protocorms stage (C), shoots initiation (D), shoot and foliage formation (E), roots formation (F)

Figure 5. The Seedlings of *S. appendiculatus* derive *in vitro* culture grow in the pot with fern medium

CONCLUSION

Propagation of *S. appendiculatus* by *in vitro* seeds germination was successfully achieved. The best medium for germination of *S. appendiculatus* was KnC medium supplemented with 30% CW and the best medium for seedling development was 1/2MS medium supplemented with 40% CW. The plantlets of *S. appendiculatus* were resulted from these research approximately more than 1500 individuals. The fully expanded seedlings were successfully transferred to perforated plastic pots.

REFERENCES

Anjum S, Zia M, Chaudhary MF (2006) Investigation of different strategy for high frequency regeneration of Dendrobium malones 'Victory'. *Afr. J. Biotechnol.* 5(19), 1738-1743.

Arditti J, Ghani AKA (2000) Numerical and physical properties of orchid seeds and their biological implications. New Phytol. 145, 367-421.

Batygina TB, Bragina EA, Vasilyeva VE (2003) The reproductive system and germination in orchids. Acta Biologica Gracovensia Series Botanica 42(2), 21-34.

Bhadra SK, Hossain MM (2003) *In vitro* germination and micropropagation of *Geodorum desinforum* (Lam.) Schltr., an endangered orchid species. Plant Tissue Cult. 13(2), 165-171.

Falsetto S (2008) An introduction to orchid flowers the definition, distribution and evolution of Orchids. http: //www.suite101.com/content/an-introduction-to-orchid-flowers-a79447 (Access 1/31'2011).

Hossain MM (2008) Asymbiotic seed germination and *in vitro* seedling development of *Epidendrum ibaguense* Kunth. (Orchidaceae**).** *Afr. J. Biotechnol.* 7 (20), 3614-3619.

Kalimuthu K, Senthilkumar R, Vijayakumar S (2007) *In vitro* micropropagation of orchid, *Oncidium* sp. (Dancing Dolls). *Afr. J. Biotechnol.* 6(10), 1171-1174.

Khayri JMA, Huang FH, Morelock TE, Busharat TA (1992) Spinach tissue culture improved with coconut water. Hort. Science 27, 357-358.

Kitsaki CK, Zygouraki S, Ziobora M, Kintzios S (2004) *In vitro* germination, protocorm, and plantlet development of mature versus immature seed from several *Ophrys* species (Orchidaceae). Plant cell reports 23(5), 284-290.

Kusumoto M (1980) Effects of coconut milk, agar, and sucrose concentration, and media pH on the proliferation of *Cymbidium* protocorm-like bodies cultured *in vitro*. *J. Japan Soc. Hort. Sci.* 48(4), 503-509.