ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology, Volume 1 Number 2-4: 111-115, 2002

In vitro Seed Propagation of Dendrobium (*Dendrobium transparens*) Orchid as Influenced by Different Media

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Abstract: Four different media namely- Hyponex, Murashige and Skoog (MS), OKF_1 and Knudson C (KC), were tested for large scale multiplication of sympodial native orchid, *Dendrobium transparens* Wall. via seeds. MS medium was found to be best for characters studied in *Dendrobium transparens*, followed by Hyponex medium but OKF_1 medium gave the least performance. Days required to seed germination was the minimum (50 days) in MS medium while the maximum days (59 days) was required by OKF_1 medium. Considering other characteristics, such as days required to protocorm formation and plantlet development, number of leaves and roots per plantlet, plantlet height and root length and finally plant survivability percentage, MS medium showed significantly better performance for *in vitro* seed propagation of *Dendrobium transparens*.

Key words: *In vitro* seed propagation, orchid, *Dendrobium transparens*

Introduction

Orchids are an excellent item for garden and for indoor decoration. They exhibit a wide range of diversity in form, size, colour and texture of flowers beyond the imagination of human mind (Mukherjee, 1983). Among the flowering plants more than 25000 species and 700-800 genera are under orchidaceae family (Begum, 2000). Several orchids have been used as food in different parts of the world. The stem of *Dendrobium* species are used in making baskets in the Philippines, Indonesia and New Guinea and pseudobulbs of *D. tokai* are used as oral contraceptives (Bose and Bhattacharjee, 1999). Commercial cultivation of orchids both for plant sale as well as cut- flower production has been developed into sizeable industries in many countries and the sale of flower runs in millions of dollars. The orchid flower trade bring about over hundred million dollars a year and the income from the sale of orchid plants is estimated as over two hundred million dollars a year (Singh, 1998). In Bangladesh, the orchid trade is still in its nascent stage. But there is a great scope of orchid trade in Bangladesh considering their availability and climatic conditions.

Orchids grow in nature through seeds but in absence of appropriate hosts they don't germinate in adequate numbers, so it remains as a rare species. These obstacles may be over come by adopting tissue culture technique. For appropriate germination of orchid seeds, *in vitro* propagation is imperative for

multiplication rather than *in vivo* (Arditti, 1979). A good number of orchids with beautiful flowers are available in Bangladesh. Like other countries, appropriate propagation techniques for large-scale production of native orchids may be a profitable source of earning foreign exchange in Bangladesh. But, there is no well-recognized report on standardization of *in vitro* suitable techniques for seed propagation of native orchids. Besides the media reported earlier were suitable for exotic varieties of orchids, which may not be suitable for our native orchids. *Dendrobium transparens* is one of the important fascinating native orchid. Therefore, the present investigation was undertaken to develop a low cost commercially applicable medium for our native orchid, *Dendrobium transparens*.

Materials and Methods

Studies on the seed propagation of native orchid species, *Dendrobium transparens* were carried out in the Tissue Culture Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during February, 1998 to May, 1999. Seeds of the mentioned orchid species were collected from Khrishibid Orchid and Cactus Nursery, Khamarbari, Dhaka through personnel contact. Four different media were used as treatment and these were:

M_1 = Hyponex medium (3 g L^{-1} Hyponex +8 g L^{-1} peptone + 2 g L^{-1} charcoal + 8 g L^{-1} Agar + 30 g L^{-1} Sucrose)
M_2 = MS medium, Murashige and Skoog (1962)
M_3 = OKF_1 medium (3 g L^{-1} OKF_1 +8 g L^{-1} Peptone + 2 g L^{-1} Charcoal + 8 g L^{-1} Agar + 30 g L^{-1} Sucrose)
M_4 = Knudson C medium (Knudson, 1964), used as check.

Completely randomized design (CRD) was followed with ten replication. Pods were collected and surface sterilization was done by 1% $HgCl_2$, 70% ethanol and dissected inside the clean bench and collected seeds were sown on to the medium. The explanted seeds were incubated at 22° C under 16 h photoperiod illuminated with fluorescent light of 3000 lux. The developed plantlet was transferred into test tube and sub culture was done as and when necessary. After the development of plantlets (Plate 1a), NAA (1mg L^{-1}) also applied in each medium except check. Data on percentage of seed germination, required days to seed germination, protocorm and plantlet formation; length of plantlet, number of roots and leaves at different dates and also plant survivability percentage were recorded, analyzed statistically and means were separated using DMRT.

Results and Discussion

***In vitro* seed germination**: There was a highly significant variation in percent seed germination among different media (Table 1). Germination of seeds in different media were shown in Plate 1b. The highest seed germination (78%) was observed in MS medium, which was followed by Hyponex medium (73%) while OKF_1 gave the poor performance (58%). This result is in agreement with that of Ismat (1982) who conducted an experiment with *Dendrobium pierardii* on MS and Hyponex media and observed

percentage of seed germination was 79 and 70 respectively. More or less similar results (percentage of seed germination was 81 and 74 in MS and Hyponex media respectively) were found by Hoque (1993) in case of *Dendrobium formusum*.
The present work is differed from Ismat (1982) and Hoque (1993) by orchid species only. The contents of each media were same in all cases. But NAA (1 mg L^{-1}) was applied in each media after plantlet development in the present study.

Required days to seed germination: Highly significant variation among different media had been observed in required days to seed germination (Table 1). Maximum days (59 days) to seed germination was recorded by OKF_1 medium while the least period (50 days) was required by MS medium. Ismat (1982) and Hoque (1993) found that required days for seed germination of *Dendrobium* sp. in MS medium is 51 and 55 days respectively. The present findings have similarity with their results.

Days required to protocorm formation and plantlet development: The required days to protocorm formation and plantlet development were significantly influenced by different media (Table 1). In case of protocorm formation, maximum duration (48 days) was required by OKF_1 medium which was significantly similar to Knudson C medium (43 days) while MS medium took the least period (36 days). Similar trends were also observed in planlet development (Table 1). Hoque (1993) stated that protocorms differentiated into plantlets within 72-78 days in different media which is closely related to the present findings. Developed protocorms in different media were shown in Plate 1c.

Plantlet height: Highly significant variation among different media at different dates had been observed

Table 1: Effects of different media on percentage of seed germination and required days to seed germination, protocorm formation and plantlet development of *Dendrobium transparens*

		Days required to		
Treatments	% Seed germination	Seed germination	Protocorm formation from seed germination	Plantlet development from protocorm formation
M_1 (Hyponex)	73a	55ab	39bc	83bc
M_2 (MS)	78a	50b	36c	79c
M_3 (OKF_1)	58b	59a	48a	92a
M_4 (Knudson C)	66a	56ab	43ab	86ab
CV(%)	4.46	4.36	6.14	2.70

Different letters indicate significant ($p \leq 0.01$) variation

Table 2: Effect of different media on plantlet height (cm) and number of leaf per plantlet at different weeks after plantlet development (WAPD)

	Plantlet height (cm) at WAPD				Number of leaves per plantlet at WAPD			
Treatments	3	6	9	12	3	6	9	12
M_1 (Hyponex)	1.57b	2.36b	2.97b	3.59b	3.00ab	4.20a	4.90ab	5.50b
M_2 (MS)	2.22a	2.92a	3.40a	4.06a	3.50a	4.60a	5.40a	6.40a
M_3 (OKF_1)	1.26c	1.68d	2.06d	2.52d	1.70c	3.10b	4.00c	4.50c
M_4 (Knudson C)	1.34c	1.97c	2.44c	2.96cb	2.40bc	3.40b	4.70b	5.20bc
CV(%)	10.07	10.10	10.14	8.60	20.80	14.60	10.70	11.10

Table 3: Effects of different media on number of roots per plantlet at different weeks after plantlet development

Treatments	At 3 WAPD	At 6 WAPD	At 9 WAPD	At 12 WAPD
M_1 (Hyponex)	2.10b	3.30b	4.40b	5.40b
M_2 (MS)	3.20a	4.40a	6.10a	7.80a
M_3 (OKF_1)	1.10c	1.90c	2.30c	3.00c
M_4 (Knudson C)	1.60b	2.40c	4.20b	5.30b
CV(%)	20.00	15.50	12.90	10.80

Different letters indicate significant ($P \leq 0.01$) variation

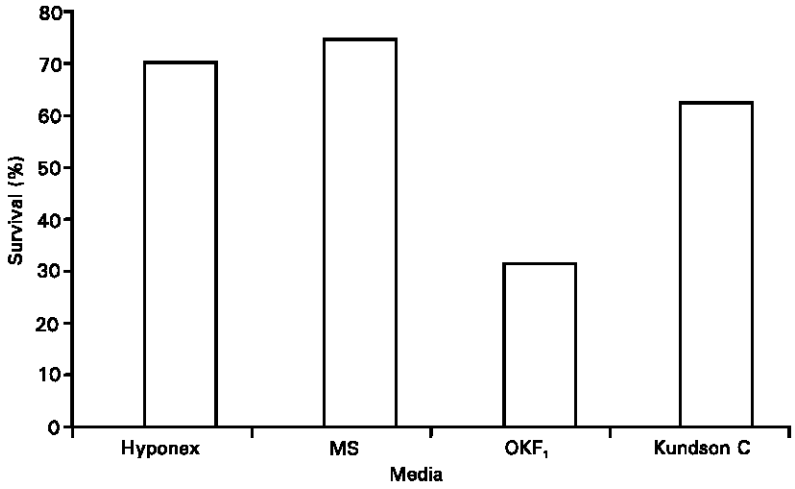

Fig. 1: Effect of different media on survivility (%) at 12 weeks after plantlet development

in plant height (Table 2). The tallest plantlets (2.22, 2.92, 3.40 and 4.06 cm at 3, 6, 9 and 12 WAPD respectively) were observed in MS medium, followed by Hyponex medium while OKF_1 medium produced the dwarf plantlets (1.26, 1.68, 2.06 and 2.52 cm at 3, 6, 9 and 12 WAPD respectively).

Number of leaves: The number of leaves per plantlet was significantly influenced by different media

(Table 2). The highest number of leaf was observed in MS medium which was statistically similar to Hyponex medium at different dates except at 12 WAPD and the least number was found in OKF_1 medium at all counting dates. This result is in agreement with that of Davidson (1994) who stated that the number of leaves on the plantlet of *Dendrobium* species showed better development in MS medium than Knudson C medium.

Number of roots: At all dates, number of roots per plantlet were significantly higher in MS medium which was followed by Hyponex medium while OKF_1 produced the lowest number of leaves (Table 3) which is consistent with that of Davidson (1994). Root length was also measured at 12 WAPD. The MS medium gave the longest root (1.40 cm) which was statistically similar to Hyponex medium (1.20 cm) while OKF_1 gave the shortest root (0.50 cm).

Plant survivility percentage: Plant survivility percentage also showed significant differences among treatments at 12 WAPD (Fig. 1). The maximum survival plant (76%) was observed in MS medium which was statistically similar to that of Hyponex medium (71%) whereas OKF_1 medium gave the lowest survival percentage (30%).

From the above discussion, it may be suggested that the MS medium was better for *in vitro* seed propagation of *Dendrobium transparens*.

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