In *vitro* Seed Germination and Seedling Development of the orchid *Dendrobium moschatum* (Buch.Ham) Sw. (Wah-so-pan)

Yin Yin Sint[1]

Abstract

Dendrobium moschatum Sw. (War-So-Pan) is one of the flowering plants belong to the family Orchidaceae. It is also known as War- So- Pan in Myanmar cultivated for its ornamental and medicinal purposes. *Dendrobium moshatum* was propagated *in vitro* by using green pod culture method in the present study. In this experiment, solid media of VW (Vacin and Went, 1949) were used for seed germination. At the end of one-week culture did not show any germination sign. Within the two weeks of culture the undifferentiated tissues of embryo of the seeds had enlarged an occupied the whole seed coat. In the thirds weeks, the seed coats become much larger than the embryo or only as large as the embryo. In the fourth weeks, the embryo stretches the coat until the latter forms a netlike enclosure around the embryo. At the fifth weeks, the seed coat disappears and the embryo globular or irregular-shaped. At sixth weeks, group of papillae develop on the bottom half of the embryo. At the seventh weeks, protocorms may be smooth or irregular and some may produce multiple buds on their surface. In the eighth week, the shoot apex appears. In the ninth weeks, the second foliaceous appear.

Key words: *Dendrobium moschatum,* Vacin and Went medium, green pod culture

Introduction

The selected plant, *Dendrobium moschatum* (Buch.-Ham.) Sw. belonging to the family Orchidaceae was widely distributed in Myanmar. There are 1600 species of *Dendrobium* in the Orchidaceae family. *Dendrobium moschatum* (Buch.-Ham.) Sw., the musky- smelling *Dendrobium*, is a species of orchid. It is native to the Himalayas (northern and eastern India, northern Bangladesh, Nepal, Bhutan, Assam, Yunnan) and Indochina (Vietnam, Thailand, Myanmar, Laos, Cambodia) (Hossain, 2002). The leaves and stems of a number of *Dendrobium* species (orchidceae) are used as health foods and nutrients (Bao, Shun and Chen, 2001).

This plant is usually propagated by vegetative method which is very slow process. The pod of this orchid contains about million of tiny seeds that contain naked undifferentiated embryos composed of 80 - 100 cells without any functional endosperm. Generally, orchids are propagated through seeds. Orchid seeds are unique in several respects. They are minute and have limited food reserves. Orchids can be classified into three categories on the basis of germination of seeds: (1) readily germinate under asymbiotic condition; (2) more difficult to germinate asymbiotically and may require special medium and culture conditions for symbiotic germination; (3) those which do not germinate symbiotically and rely on symbionts for germination. The flower is pollinated when the pollinia are placed on the stigma of an orchid flower. If successfully fertilization take place, the ovary beneath it also begins to grow, and soon attains its full size. In the case of *Dendrobium moschatum*, it is cylindrical, green, and hanging downwards. When it is ripe, the side gape open with three slits. And the seeds are free to fall out, which they do at any puff of wind. Each seed consists of a tiny round embryo surrounded by a loose translucent coat, and so has low density and consequently a slow rate of falling. Orchid seeds very much in the details of their size, the shape of the seed coat, and their color, but all are very small. Both commercial and amateur growers need appropriate media, which insure a high percentage of germination and rapid seedling growth. The impaired germination of the mature seeds has also been correlated with the quality of their food reserves. The

[1] Dr.,Associate Professor, Department of Botany, Taungoo University

various culture media devised by earlier author with some modifications wherever necessary have been employed at various stages of culture. The present investigation is meant for rapid mass propagation of *Dendrobium moschatum* by asymbiotic *in vitro* green pod culture technique which, then, can be exploited for their conservation.

Material and methods

Plant materials

Dendrobium moschatum (Buch.-Ham.) Sw. was obtained by from Myanmar Agriculture Service, Yangon.

Pollination and harvesting

Healthy flowers of desired size and shape were selected for pollination. Pollinia from one flower were brought into contact with the stigma of the same flower visited, adhering to the sticky substance on the stigmatic surface. Thus, the fertilization achieved. The sepals and petals then become wilt and wither after pollination. After that the ovary gradually increased in size. Three months after pollination, the green capsules were harvested.

Surface sterilization

Three-months old green capsules of *Dendrobium moschatum* (Buch.-Ham.) Sw. were washed under running tap water and Teepol for three times. Final disinfection was done by submerging the capsules in 70% ethanol for 30 min and 10 min in 10% clorox and washing thoroughly with double sterile distilled water.

Preparation of media

The media were prepared according to Arditti (1992). The distilled water (50 ml) was poured into beaker (500 ml size). Then the stock solutions were added to the beaker. Before the gelling agent were added to the prepared solution, the solution was adjusted to the pH of the medium was adjusted at 5.0 ± 0.1 using 1N NaOH or 1N HCl solution. The total volume of solution was made up to 200 ml and it was gently heated and stirred until agar was completely dissolved. Then the prepared medium was equally distributed to the bottles. They were autoclaved at 1.05kg pressure and 121ºC for 15 minutes. Finally, culture bottle containing medium were cooled and labeled for the culture of *Dendrobium moschatum*.

Asymbiotic culture

The sterilized capsules were flamed and split longitudinally with a sterile surgical blade. The powdered seeds were inoculated on the surface of agar gelled nutrient medium (VW- Vacin & Went, 1949).

Study of asymbiotic germination

Once the spherules were formed, observations were recorded at an interval of one week to trace different stages of developed of protocorms. Each observation was using an Olympus microscopes.

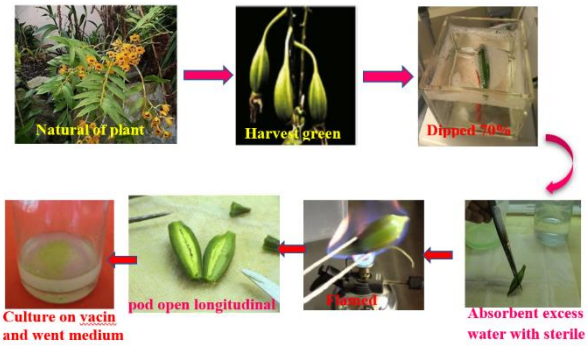

Fig. 1 procedure of green pod culture

Results

Asymbiotic germination

Seeds from green capsule of *Dendrobium moschatum* were aseptically grown in VW medium. At the time of sowing, the seeds were fusiform and each contained an ellipsoidal embryo. The seed coat, derived from the outer integument, is composed of elongated cells (Fig 2)

Fig. 2 Seeds of *Dendrobium moschatum* observed before culture

After sowing seeds in culture, germination i.e developmental stages of embryo to protocorm were observed weekly. Within two weeks of culture, each seed containing undifferentiated embryo slightly swelled up by imbibing waters and nutrients (Fig 3).

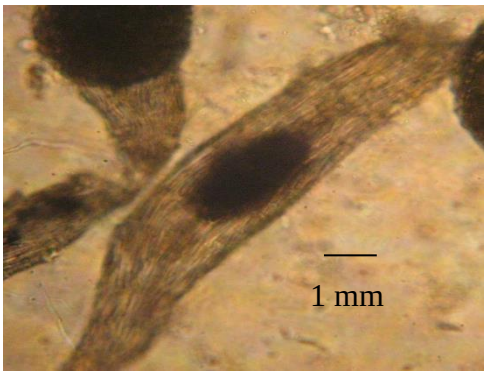

Fig. 3 Seed containing embryo of *Dendrobium moschatum* observed at 2nd week of culture

At the 3rd weeks, embryo apparently swelled up and turned to globular structure (spherules). By this time embryo had enlarged and occupied the whole seed coat spherical shape and apparently swelled embryo of *Dendrobium moschatum* observed at 3rd week of culture (Fig 4).

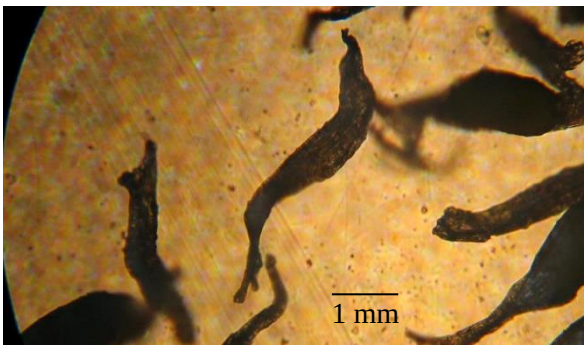

Fig. 4 Spherical shape and apparently swelled *Dendrobium moschatum* embryo observed at 3rd week of culture.

At 4th week, as the seedling enlarges, the seed coat brusted and the embryo were ruptured from the seed coat (Fig. 5).

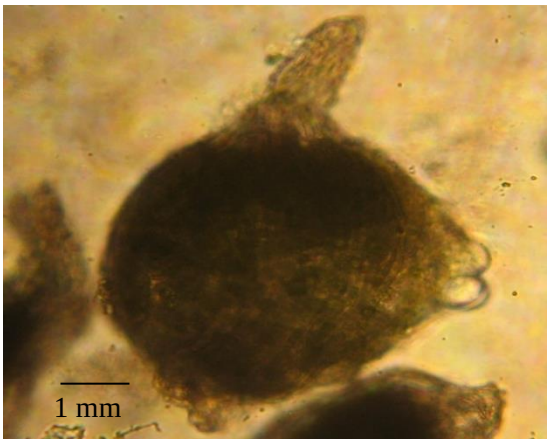

Fig. 5 The embryo with broken seed coat

At the end of 5th week, due to repeated cell divisions and increased in size of embryo, at the end of 5th week, it reptured from the testa. Then the seed coat disappears and papillae emerged from it (Fig. 6).

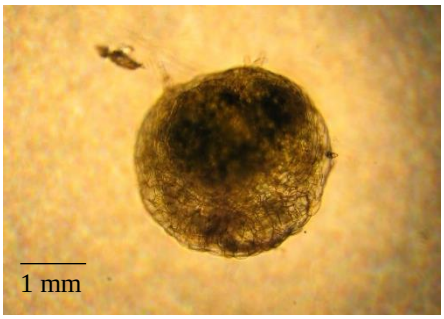

Fig. 6 Developed embryo without seed coat having papillae observed at fifth week.

At the 6th week, group of papillae develop on the bottom half of the embryo. They are fine white hair quite visible to the naked eyes. The structure with the hair at the basal part become protocorms. The upper part of the protocorm formed the vegetative apex (Fig. 7).

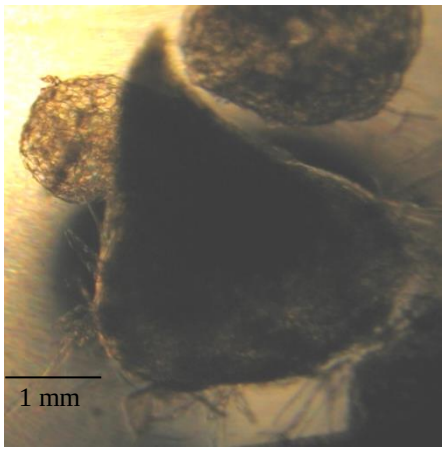

Fig. 7 Developed embryo with papillae and developing shoot apex observed at 6th week.

At 7th week, embryo developed into early stage of protocorm which may be smooth or irregular and some produced minute-hairs. The upper part of the protocorm formed the vegetative apex of the stem with a leaf primordium and continues to enlarge. (Fig. 8).

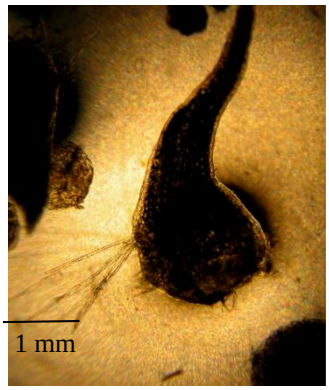

Fig. 8 The early stage of protocorm having minute hairs observed at 7th week.

As the shoot organs were formed, it becomes asymmetrical body which produced very minute leaves. The second foliaceous primordial appeared, opposite and a certain distance from the first, thus delimiting the vegetative of the stem (Fig. 9).

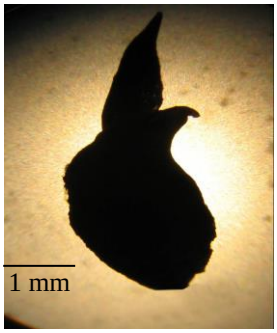

Fig. 9 A protocorm with shoot apex with very minute leaves observed at 8th week.
As the leaf primodium keeps growing, at the 9th week, an opening form by the edges of the ridge gradually moves to a lateral position (Fig. 10).

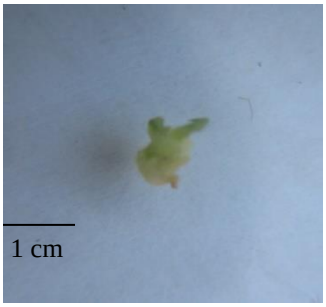

Fig. 10 A protocorm with shoot with minute leaves and no roots but some hairs (shootted protocorm) observed at 9th week.

Table 1.1 Seed germination percentage of *Dendrobium moschatum* in culture

Duration (week)	Germination seed per replicate (%)			
	ungerminated seeds (a)	number of seeds started to germinate (b)	Protocorm without shoot (c)	protocorm with shoot (d)
1st	100	-	-	-
2nd	81.2	18.8	-	-
3rd	69.8	30.2	-	-
4th	63.4	36.6	-	-
5th	53.8	46.2	-	-
6th	37.8	18.6	43.6	-
7th	24.2	18.6	57.2	-
8th	16.6	13.2	58.8	13.4
9th	7.4	4.6	67.4	19.2

Mean values of 5 replicates. The germination percentage was calculated with the following formula:

$$\text{Percentage of seed germination} = \frac{100\,(b + c + d)}{a + b + c + d}$$

a = ungermination seeds, b = seed coat rupture

c = protocorms without shoot, d = protocorms with shoot

The method of calculating germination percentage is according to the method of Pierik *et al.*, 1988.

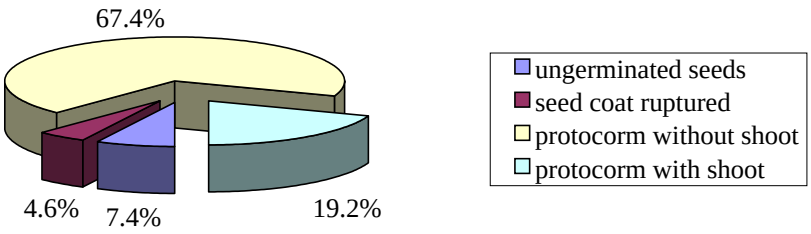

Fig. 11 Seed germination of *Dendrobium moschatum* at 9th week

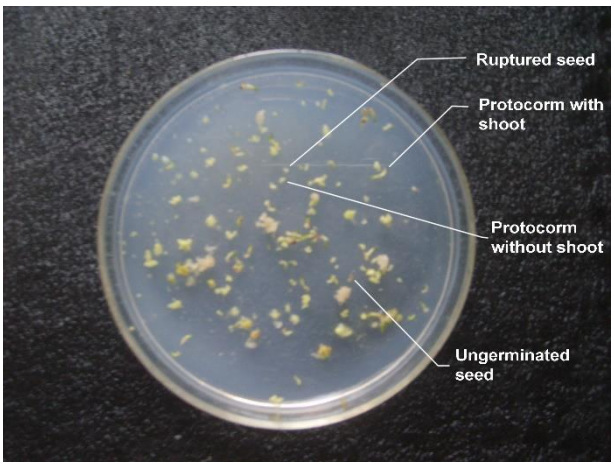

Fig. 12 Seed germination of *Dendrobium moschatum* in 9th week

Percentage of seed germination in every week were as shown in Table 1.1. In the 1st week, no germination of *Dendrobium moschatum* seeds were observed. In the second week, 81.2% of seed did not germinate, however, 18.8% of seed showed rupture of seed coat. In the third week, ungerminated seeds were 69.8% while 30.2% showed that embryo slightly enlarged but seed coat did not rupture. In the fourth week, ungerminate seeds was 63.4% and 36.6% of seed started to germinate but seed coat did not apparently break. In the fifth week, ungerminate seeds were 53.8% and the rest 46.2% had seed coat ruptured. In sixth week, 37.8% of seeds did not germinate, 18.6% started to germinate and the seed coat ruptured while 43.6% of seeds developed into protocorms. In seventh week, 24% of the seeds did not germinate, 18.6% of seeds showed germination without rupture from seed coat and 57.2% of seed developed into protocorm without shoot. In eighth week, 16.6% of seeds showed no germination and 13.2% was seed coat ruptured, 58.8% of seeds in which embryo develop into protocorm without shoot and 13.4% was protocorms with single shoot. At the end of ninth week, it was observed that 7.4% of seed did not germinate and 4.6% slightly germinated but the embryo did not rupture from seed coat. Protocorms without shoot was 67.4% while 19.2% of seed differentiated into protocorm with shoot at the end of ninth weeks. This result are indicated that Table 1.1 and Figure 11 and 12.

Discussion and conclusion

Dendrobium moschatum was propagated *in vitro* by using green pod culture method in the present study. In this experiment, solid media of VW (Vacin and Went, 1949) were used for seed germination. The seeds germinated rapidly in aseptic culture. At the end of one-week culture did not show any germination sign. Within one month after germination, many embryos from seeds become swollen and green. These findings agree with the report of Minea *et al*., (2004). In the sixth and seventh week, they even produced protocorm without shoot tips. In eighth and ninth weeks, protocorm produced shoot tips. Similar finding was also reported by Minea *et al*., (2004). In the embryo and development stage of protocorm, the seeds (initial stage) were brightly yellow, seed testa to the *Dendrobium* type (Dressler, 1993) (Fig. 2). Within the two weeks of culture the undifferentiated tissues of embryo of the seeds had enlarged an occupied the whole seed coat (Fig. 3). This result agrees with the finding of Buyun *et al*., 2004. In the thirds weeks, the seed coats become much larger than the embryo or only as large as the embryo (Fig. 4). Similar result has been reported by Teoh (1980) who state that the embryo become pear-shaped. In the fourth weeks, the embryo stretches the coat until the latter forms a netlike enclosure around the embryo (Fig. 5). At the fifth weeks, the seed coat disappears and the embryo globular or irregular-shaped (Fig. 6). This finding agrees with Withner (1959). At the sixth weeks, group of papillae develop on the bottom half of the embryo (Fig. 7). This statement agrees with Teoh (1980) who reported that the papillae are fine white quite visible to the naked eye. At the seventh weeks, protocorms may be smooth or irregular and some may produce multiple buds on their surface (Fig. 8). This agrees with Batygin *et al*., (2003). During the protocorm formation, the basal part functioning as a storage organ, consist of homologous to the hypocotyls the embryo of flowering plants (Teryokin, 1977). Early in germination, chlorophyll appears in the protocorm cell of epiphytic orchid (Batygina *et al*., 2003). In the eighth week, the shoot apex appears (Fig. 9). This finding agrees with Hossain (2008). The apical part to the protocorm, consisting of small number of cells formed a “tubercle” which turns into shoot apex. In the ninth weeks, the second foliaceous appear (Fig. 10). This

finding agrees with Hossain (2008). As the primordial keeps growing, an opening formed by the edges of the ridge gradually moves to a lateral position. Interpretation of the first foliar organ of orchid differ. Teryokhin & Nikiticheva (1968) stated that not only the first but also the second appendicular organs formed in the protocorm are cotyledons.

In conclusion, *in vitro* propagation of orchids through seeds can produce large number of orchids in reasonable short time. The present study was undertaken to develop an efficient protocol for *in vitro* propagation of *D. moschatum* through seeds and ultimately assist in its conservation.

References

Arditti, J. (1992). *Fundamentals of orchid biology.* New York: John Wiley and Sons Inc.

Bao, X.S., Shun, Q. S. and Chen, L.Z. 2001. The medicinal plants of Dendrobium (Shi –hu) in China, A coloured atlas, Sanghai; Press of Fudan University and Press of Sanghai Meidcal University

Batygina, B., T. Bhadra, S. K. & Vasilyeva, V. E. 2003. The reproductive system and germination in orchids. *Act a Biologica Cracoviensia Series Botany 45 (2)*, 21 –34.

Buyun L. Lavrentyva A, Kovalska L. Lvannikov R. 2004. *In vitro* germination of seeds of some rare tropical orchids. *Acta Universitatis latviensis, Biology, 676*, 159 – 162.

Dressler, R. L. 1993. Phylogeny and classification of the orchid family. Portland: Dioscarides Press.

Hossain, M. M. 2008. Asymbiotic seed germination and *in vitro* seedling development of *Epidendrum lbaguense* Kunth. (Orchidaceae). *African Journal of Biotechnology*. *7* (20), pp. 3614 – 3619.

Hossain, A.B.M. 2002. A Taxonomic report on the genus *Dendrobium* Sw.. Bangladesh Journal of Plant Taxonomy 9(2): 47-55.

M Minea, C Piluek, A Menakanit. 2004. A Study on Seed Germination and Seedling Development of S*pathoglottis* Bl. Orchids Mao Minea1, Chitrapan Piluek1, Alisara.

Teoh, E. S. (1980). *Asian Orchids.* Singapore: Time Books International, Time Center.

Teryokhin, E.S, Nikiticheva ZI. 1968. Post - seminal development of parasitic Angiospermae. I. Metamorphosis. *Botanical Cheskii Zhurnal SSSR 53*, 39 – 37.

Teryokhin, E. S. 1977. *Parasitic flowering plants Evolotion of Ontogenesis.* Leningrad: Nauka.

Vacin, E. & Went, E. 1949. Culture solution for orchid seedling. Botany Gazart 110, 605 – 613.

Withner, C. L. 1959. Orchid Physiology. In: C. L. Withner (Ed.). The Orchid: A scientific survey. New York: Ronald Press.