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IN VITRO SEED GERMINATION AND DEVELOPMENTAL MORPHOLOGY OF SEEDLINGS IN *DENDROBIUM OVATUM* (L.) KRAENZL.

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Abstract

In vitro seed germination, protocorm development, organization of shoot apical meristem, and initiation of leaf and root primordia are described in *Dendrobium ovatum* (L.) Kraenzl. The first leaf primordium was initiated opposite to the cotyledonary sheath on the apical meristem. The subsequent leaves arising from the apical meristem were alternate, resulting in two ranked condition of leaves, in the young seedlings. The origin of the first lateral root near the lower end of the procambium was observed. The terminal position of the epicotyl and absence of primary root has been established. The germination of orchid seed was of the epigeal type.

Introduction

BERNARD (1899, 1902, 1909) AND Burgeff (1909) pioneered the studies on *in vitro* methods of seed germination by making the use of symbiotic fungus. Knudson (1925), for the first time, demonstrated that the orchid seeds could be germinated *in vitro* without the symbiotic fungus. Similar studies were carried out by Clement (1924a,b, 1929) and Knudson (1930, 1941, 1946, 1950, 1951, 1952). Withner (1959) has reviewed and compiled most of the information about different aspects of *in vitro* culturing of orchids. Following the improvement in the culture techniques and formation of defined chemical nutrient media, immature embryos could be easily grown into seedlings (Anuprabha and Pathak, 2012; Arora *et al.,* 2016; Bhatti *et al.,* 2017; Borah *et al.,* 2015; Chauhan *et al*., 2010; Decruse and Gangaprasad, 2018; Ito, 1955; Kaur *et al.,* 2017; Lekshmi and Decruse, 2018; Mohanty and Salam, 2017; Nimoto and Sagawa, 1961, 1962; Pathak *et al*., 2001, 2011, 2016, 2017; Rao and Avadhani, 1963, 1964; Sagawa, 1962; Sagawa and Valmayor, 1966; Sibin and Gangaprasad, 2016; Valmayor *et al*., 1977; Verma *et al*., 2015; Withner, 1943, 1955). The details of seedling development from the point of histogenesis and organogenesis have been reported in a few orchids, although a considerable number of publications are available describing exomorphic characters of protocorms and seedlings (Arditti, 1966; Arekal and Karanth, 1980; Batygina and Vasilyeva, 1983; Hossain *et al*., 2013; Mathews and Rao, 1985; Mohanty and Salam, 2017; Muralidhar and Mehta, 1985; Nishimura, 1981; Pathak *et al.,* 2011; Shushan, 1959).

The earlier workers like Bernard (1909), Carlson (1943), Mitra (1971), Pfitzer (1877), and Rao (1964, 1967) have interpreted the structure of orchid embryo in different ways. The opinions expressed regarding the morphology of germinating seeds are at variance. The apical part of the embryo in the seeds of *Dendrobium glumaceum* has been considered as cotyledon (Pfitzer, 1877). However, Carlson (1935, 1943) opined that the posterior food-storing zone of the embryo is cotyledon. A classification of orchid embryo into two types, namely *cotyledonous* and *acotyledonous* forms have been proposed by Veyret (1974). According to her, in the former type, the cotyledon is terminal while in the latter, the epicotyl is terminally situated. Other investigators like Harrison and Arditti (1978), Mitra (1971), and Rao (1963) indicated that the first leaf is cotyledon. On the other hand, Rangaswamy (1967) and Veyret (1974) are of the opinion that the orchid embryo possesses no cotyledons, nonetheless, germination brings about meristem formation and differentiation. Thus, there is confusion with regard to the morphology of germinating embryo and an acceptable opinion is yet to emerge. This status of the embryo part, is due to the lack of detailed information on the subject dealing with the sequential stages of histogenesis and organogenesis during seedling development. Therefore, an attempt has been made in the present study to germinate the seeds *in vitro* and find out the embryo-seedling morphogenesis. The investigation includes the morphogenetic response of germinating embryo of *Dendrobium ovatum*. The histomorphology of the embryo during seed germination and subsequent differentiation and seedling ontogeny have been described.

Material and Methods

Green capsules of *Dendrobium ovatum* (L.) Kraenzl. were obtained from plants growing in Hejamadi, Udupi, South Canara district, Karnataka state. The capsules were surface sterilized by dipping them in 0.1% solution of

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mercuric chloride for 5-10 min. They were then washed 3-4 times with sterile distilled water and surface sterilized by dipping in ethanol followed by flaming in the inoculation chamber (vertical laminar flow). The capsules were cut open with a sterilized safety razor blade. The seeds were scooped out and transferred on to the nutrient medium in the flask. The culture media used in the present investigation were: i) Modified White's medium- MW (Rangaswamy, 1961); ii) Murashige and Skoog's medium-MS (Murashige and Skoog, 1962); and iii) Prasad and Mitra's medium (for germination of seeds)- PM1 (Prasad and Mitra, 1975). The pH of the medium was adjusted at 5.5. The cultures were maintained in 12 hr photoperiod from cool daylight fluorescent tubes giving a total intensity of 2000 lux at the culture level and at temperature 25±2°C and 50-60 % relative humidity. Observations were made once in five days to find out the emergence of germinating embryo, leaf, and root. When the growth was resumed, they were transferred to fresh medium.

Germinating embryos, protocorms, and young seedlings were fixed in formalin-acetic acid-alcohol (FAA) at appropriate intervals and subsequently stored in 70% ethyl alcohol after a thorough wash in running water. Subsequently, they were dehydrated in alcohol-xylol series and embedded in paraffin wax of 58-62°C melting point. Serial microtome sections were cut at 10-14 µm thickness. The sections were stained in Heidenhain's iron-alumn haematoxlin with erythrosine in clove oil as counter stain. Appropriate stages were photomicrographed using microscope.

Results

Germination of Seeds and Seedling Development

Seeds in the green capsule ('pod') were minute, spindle shaped, and embryonate but non-endospermic. Seed coat was having reticulate ornamentation (Figs. 1-2). The seeds sown on MS and PM1 nutrient media did not show any sign of germination even after 60 days of inoculation. On the other hand, the response to germination was positive on MW medium where protocorms were produced on the same medium (Fig. 3). With the addition of coconut milk (200 mlL^{-1}) and peptone (4 gmL^{-1}) to the MW medium, the response was much better (Fig. 4). The enlargement of the embryos was the first sign of germination (Fig. 5). In about 20-25 days, the embryos turned green because of the production of chlorophyll. In the mean time, unicellular, unbranched absorbing hair arose from peripheral cells of the posterior part of embryo. Increase in size of the embryo was followed by the organization of a beak-like structure at its terminal region. By this time, the seed coat broke open vertically exposing the beak-like projection while the absorbing hair extended out through the thin seed coat at the lower end (Figs. 6-7). This stage of development is conventionally designated as the protocorm. The beak-like structure later became a scale-like sheath as the seed coat was cast off (Fig. 8). The first leaf was initiated in about 50-60 days of inoculation; it was enclosed by the earlier formed sheath but alternating to it (Fig. 9a-i). As growth continues, the subsequent leaves were formed in the same sequence so as to organize the two-ranked state in the young plant.

The first root appeared after the formation of the first leaf in the seedling (Fig. 9j). The number of roots increased as more of leaves were formed in the young plant (Fig. 10). Ten months after inoculation of the seeds, the seedlings bearing 3-5 lanceolate leaves and 1-2 roots developed the pseudobulbs. These plants thrived well in paper cups containing sterile vermiculite and fed with dilute nutrient solution in the culture room for about 60 days before being transferred to the earthen pots and kept in open.

In one of the culture flasks, ten months after inoculation of seeds, some of the plants with 2-3 leaves produced a flower which appeared dull white in colour, much smaller in size compared to the one in nature and withered after a wk (Fig. 11).

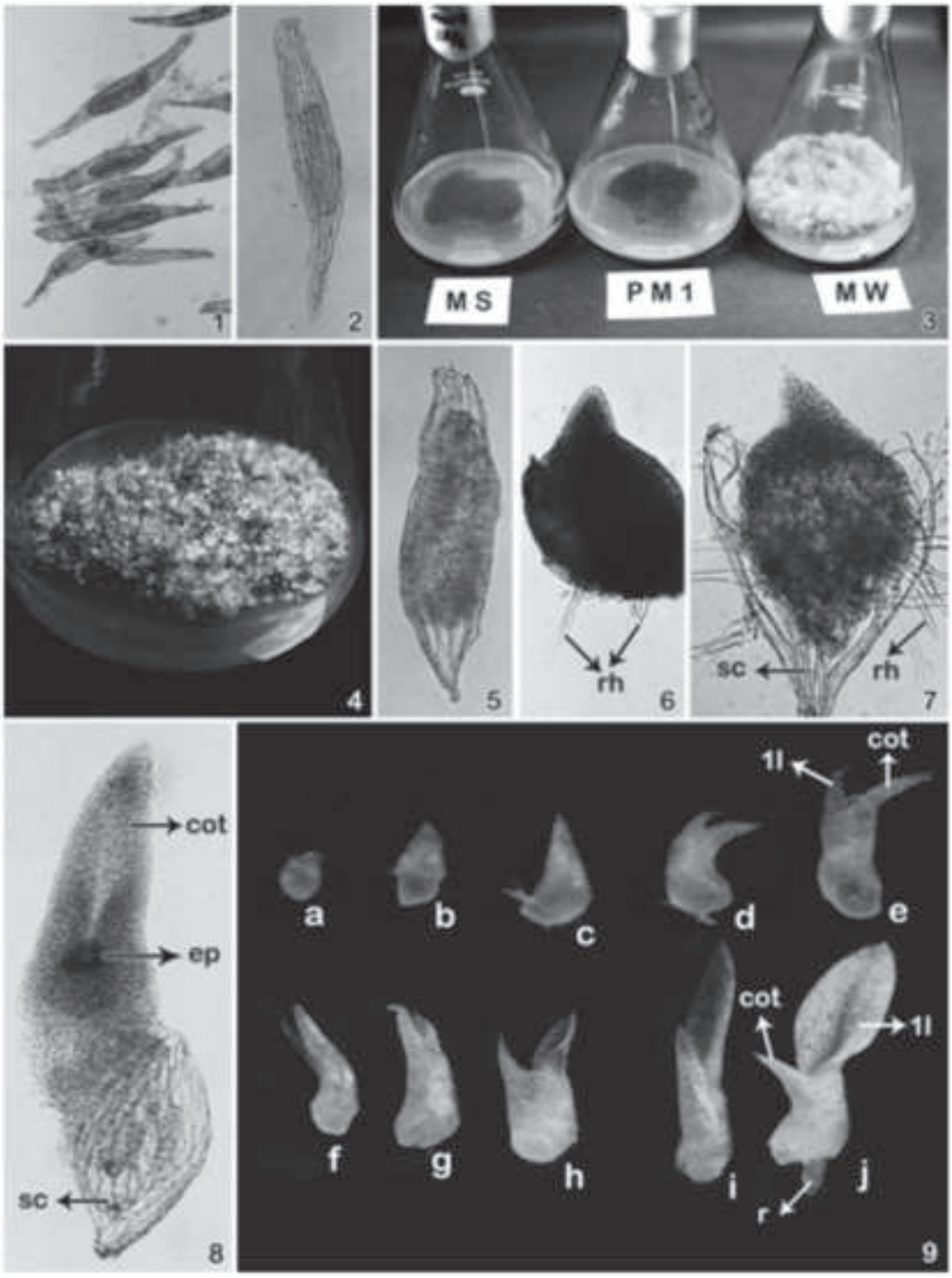
Histology and Organogenesis

The seeds at the time of sowing on to the nutrient medium enclosed of ellipsoidal embryos. As the embryo began to germinate on the nutrient medium, its cells of the radicular axis enlarged in size (Fig. 12). As a consequence, that region increased in size. Cell divisions occurred in the cotyledonary and epicotylary regions; the rate of these divisions was faster in the subsequent stages. As a result, the cotyledonary region presented a short beak-like appearance while epicotyl part became a short mound of densely protoplasmic cells (Figs. 13-14). Meanwhile, some of the epidermal cells of the radicular axis extended out in the form of narrow tubes and functioned as absorbing hairs.

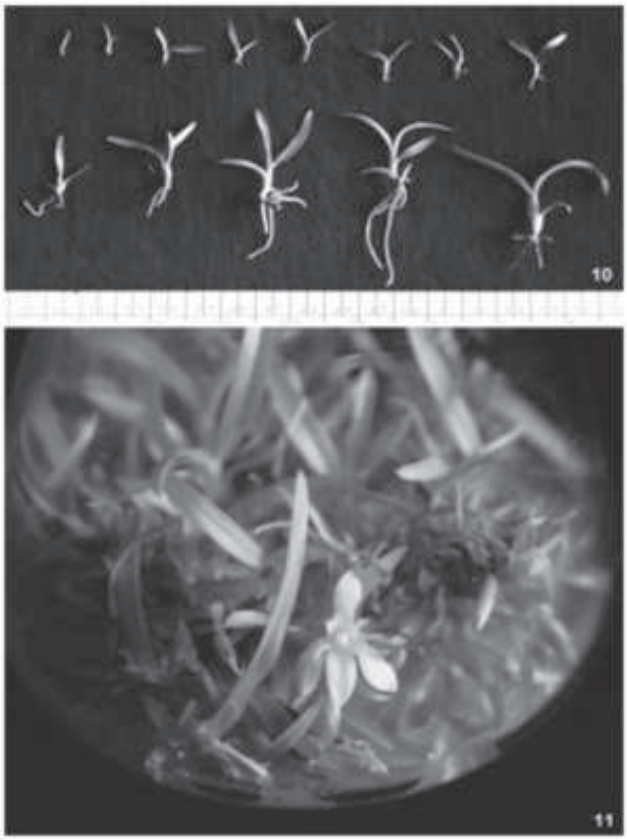
During further stages of development, there was an increase in length of the cotyledon and its basal region extended out laterally by cell division and cell enlargement forming a collar first and then, a scaly sheath enclosing the epicotyl mound. In paracotyledonary long sections of the germinating embryo, the epicotyl appeared completely over arched, giving a scope for interpreting its origin as endogenous, which is not (Figs. 15-16).

The first leaf primordium initiated from the stem tip alternating to the cotyledon (Fig. 17). As this primordium

was in the process of organizing the young green leaf, cells located below the shoot apex divided actively in the longitudinal manner and engendered a column of procambial strand of narrow elongated densely protoplasmic cells (Fig. 18). This strand extended towards a side in the radicular axis. The next leaf



Figs. 1-9. *In vitro* seed germination and seedling development in *Dendrobium ovatum*: 1, mature seed at the time of culture, ×61; 2, mature seed enlarged, ×106; 3, Seeds on MS, PM1 and MW media, note the germination response, ×0.4; 4, Seeds sown on MW medium containing coconut milk (200 mlL^{-1}) and peptone (4 gmL^{-1}) show maximum per cent of germination, ×1.2; 5, Germinating seed, note enlarged embryo, ×145; 6, Germinating embryo, note the organization of beak-like structure and production of absorbing hairs from posterior part of embryo, ×90; 7, Emergence of protocorm from the seed, ×111; 8, Young protocorm, note scale-like sheath enclosing epicotyl, ×75; 9a-i, Origin of scale-like sheath and first leaf; 9j, Young seedling with scale-like sheath, first leaf and root, ×18. (cot, cotyledon; ep, epicotyl; r, root; rh, absorbing hairs; sc, seed coat; 1l, first leaf).



Figs. 10-11. Young seedlings of *Dendrobium ovatum*: 10, Different stages of seedling development, note the number of roots increases as more leaves are formed, ×1.5; 11, Flowering plant in culture flask ×1.7.

primordium was organized on the stem tip opposite to the first leaf setting forth the process of establishing the two ranked condition of the leaves on the stem (Figs. 19-20).

The first lateral root was initiated endogenously at the lower end of the procambium. The young root pierced through the protocorm tissue and emerged out on a side of the radicular axis (Figs. 21-22). More of roots were formed endogenously as more leaves were produced on the plant. Finally, the basal large-celled, thin walled food storing cells of the old protocorm broken down and degenerated along with the absorbing hairs, as the seedling became independent by the production of more leaves and roots.

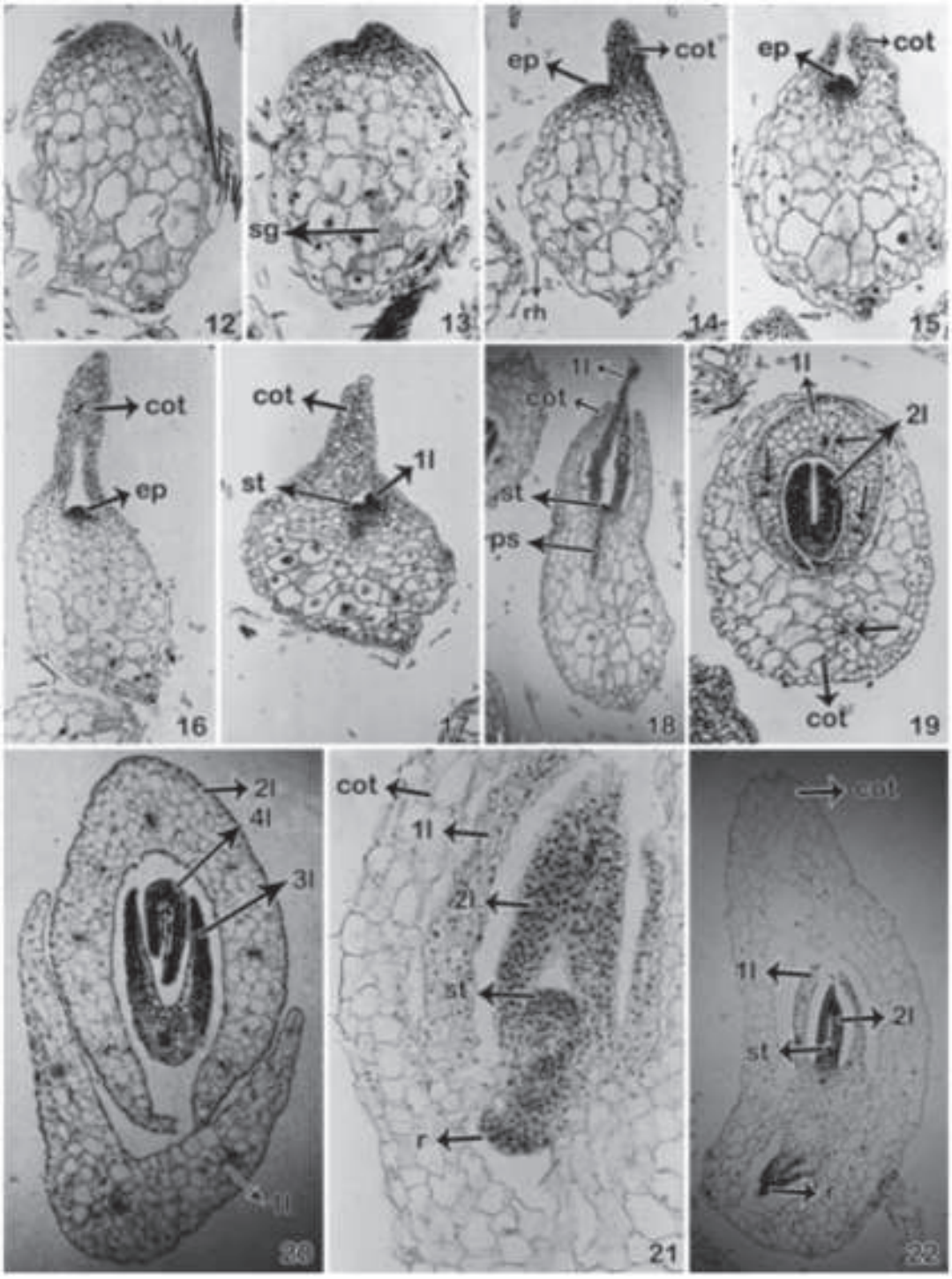
Discussion

It is well known that orchids require the association of a suitable symbiotic fungus for their seed germination in nature (Arditti, 1967; Harrison and Arditti, 1978). It has also been demonstrated that they could be germinated *in vitro* without the fungus (Anuprabha and Pathak, 2012; Arditti and Bils, 1965; Arekal and Karanth, 1978; Bhattacharjee and Hossain, 2015; Carlson, 1935; Chennaveeraiah and Patil, 1975; Clement 1924a, b; Curtis, 1943; Knudson, 1922, 1924; Mohanty and Salam, 2017; Pathak *et al*., 2011, 2016; Prasad and Mitra, 1975; Rao, 1977). Thus, the early concept that the orchid seeds are sterile and are incapable of germination (Constantin, 1913) is no more valid (Arditti, 1967). The germination of seeds of epiphytic orchids posed no problem and this has been pointed out by Mc Intyre *et al*. (1972, 1974), Stoutamire (1963, 1964a, b), and Warcup (1971) who, however, have indicated that difficulty was encountered in the germination of terrestrial orchid seeds, in contrast to those of epiphytic taxa. Stoutamire (1974) opined that the difficulty involved in the germination of seeds of terrestrial orchids is due to the fact that they are mostly endemic and are with longer duration of dormancy in comparison with the epiphytic ones. It is applicable more to the saprophytic orchids-the extreme mycotrophs. Further, he also concluded that the germination of seeds of terrestrial orchids especially that of *Listera cordata* and *Zeuxine sulcata* is difficult. Nevertheless, successful germination of seeds of *Zeuxine strateumatica* (=*Z. sulcata*) achieved by Arekal and Karanth (1978) with minimum modification of Knudson's medium showed that even in such cases, it is possible to raise the plantlets *in vitro*.

The present aspect of investigation was mainly aimed at to understand the mode of seed germination, histology and organogenesis of the developing seedlings and it was not for understanding the influence of inorganic or organic components of the nutrients or the influence of growth regulators individually. Although several nutrient media have been suggested for orchids by different researchers (Rao, 1977), three well known nutrient media were tried in the present study to investigate the germination response of seeds of *D. ovatum*. While, MW medium promoted the formation of protocorms, MS medium did not. On the other hand, PM1 medium was less encouraging. Further, the addition of coconut milk (200 mlL^{-1}) and peptone (4 gmL^{-1}) to the MW medium enhanced the percentage of seed germination. It is apparent, therefore, that different species respond differently to some of the nutrient media.

The seeds of *D. ovatum* germinated in about 25-30 days, while 30-35 days were required for the same in *Spathoglottis plicata* (Bapat and Narayanaswamy, 1977). Nevertheless, achievement of seed germination in 15 days has been reported for *Arundina bambusifolia* (Mitra, 1971), *Spathoglottis plicata* (Chennaveeraiah and Patil, 1975; Prakash and Aow, 1973) and in about 60 days for *Bromheadia finlaysoniana* (Jeyanayaghy and Rao, 1966). The time taken by the seeds to germinate *in vitro*, apparently, is determined on the type of nutrient medium, the supplements added and the taxon concerned.

The green pod culture proposed by Sagawa (1962) and Mohanty and Salam (2017) gave an increased percentage of germination in the present study as compared to the seeds of mature brown capsules.



Figs. 12- 22. Histology of *in vitro* germination of seeds of *Dendrobium ovatum*: 12, L. S. of germinating embryo, note enlarged cells at the radicular axis, ×152; 13-14, L. S. of germinating embryo, note the organization of beak-like cotyledon and dome shaped epicotyl, ×106; 15-16, L. S. of protocorm in paracotyledonary plane, note the collar-like cotyledon enclosing the epicotyl, ×125; 17, L. S. of protocorm in paracotyledonary plane, note initiation of first leaf, ×80; 18, L. S. of protocorm to show procambial strand below the stem tip, ×70; 19, Transection, upper part of the shoot apex to show alternate arrangement of sheath-like cotyledon, first leaf and second leaf; note single conducting strand in sheath-like cotyledon and three or more in leaves (indicated by arrow), ×130; 20, T. S. of seedling above the level of the cotyledon to show alternate arrangement of leaves, 21-22, L. S. of seedling, note origin of lateral root, ×133, ×14. (cot, cotyledon; ep, epicotyl; ps, procambial strand; st, stem tip; r, root; rh, absorbing hair; 1l, first leaf; 2l, second leaf; 3l, third leaf; 4l, fourth leaf).

Sagawa (1962) and Pathak *et al*. (2011) opined that the increased percentage of germination is due to the fact that during surface sterilisation, prior to inoculation, it was the surface of the capsule that was affected by the sterilising agent and not the inner seeds. However, it should be ascertained whether the seeds of the older capsules accumulate any of the chemicals that would interfere with the process of immediate germination.

On the nutrient medium, the embryos swelled many times its original size and emerged out through vertical slits of the seed coats. This observation is in conformity with the previous reports on the orchids (Carlson, 1935, 1943; Kohl, 1962; Mitra, 1971; Rao, 1967; Shushan, 1959). The absorbing hairs produced at the posterior end of the protocorms were observed in the present study. However, they develop on the entire surface of the protocorms except at the apical meristematic region in *Vanilla* (Knudson, 1950).

There appears to exist a direct correlation between absorbing hair formation and chlorophyll development in the protocorms. The enlarging embryo became green soon after sowing; this has been observed in the present study, a feature also true for *Bletilla*, *Calopogon*, *Disa*, *Pogonia,* and *Spiranthes* (Stoutamire, 1974). On the other hand, the chlorophyll development is tardy in *Dactylorhiza*, *Habenaria*, and *Ophrys* (Stoutamire, 1974).

It should be pointed out that in the present study, callus did not develop from the embryo at any stage of germination. Organization of callus has been recorded in *Vanda tricolor* (Curtis and Nichol, 1948) and *Vanda* hybrids (Goh, 1970a, b). In these instances, the investigators supplemented the medium with barbiturates or auxins along with coconut milk. Nonetheless, callus formation from germinating mature seeds even without the addition of growth promoting substances has been noted by Rao (1963). Curtis and Nichol (1948) concluded that the immature and undifferentiated stages of orchid seeds probably caused the callusing of seeds. Narayanswamy and Norstog (1964) expressed that the production of more of callus during immature seeds culture is probably due to unstable polarity within the embryo unlike the mature ones. Despite the addition of coconut milk to the nutrient medium in the present study, there was no sign of callus initiation. Further, not all species of orchids have responded similarly so far with regard to the callus production during seed germination. It is, therefore, logical to assume that different species of orchids respond differently with regard to callus development during seed germination. The present study has also demonstrated that only one protocorm is organized from an embryo of a germinating seed, although coconut milk was an ingredient of the nutrient medium.

Histology and Organogenesis

A histological examination of mature embryo of the present study revealed a terminal-basal distinction in median longitudinal section. The basal region (radicular axis) consisted of large cells with vacuolated cytoplasm gorged with reserve food materials; while the cells of the terminal part were smaller in size and lodge dense protoplasm. Furthermore, the cotyledon and epicotyl were present while typical radicle was absent. There is no controversy with regard to the absence of radicle and procambium in the mature embryo (Batygina and Vasilyeva, 1983; Carlson, 1943; Mitra, 1971; Swamy 1949). However, as an exception, Rao (1967) reported the presence of procambial cells below the shoot apex in the embryo of *Arundina graminifolia*.

In the present study, it was observed that the meristematic activity was confined to the terminal end of the germinating embryo whereas the cells of the basal part only enlarged and vacuolated as recorded earlier in *Arundina* (Mitra, 1971), *Calopogon* (Carlson, 1935, 1943), *Dactylorhiza maculata* (Batygina and Vasilyeva, 1983), *Dendrobium* (Gilliland, 1958), *Spathoglottis* (Prakash and Aow, 1973), and *Taeniophyllum* (Mutsuura *et al.*, 1962). Following this, some of the cells of the epidermis at the radicular end elongated and formed unicellular absorbing hair. The protocorm at this stage exhibited three regions: i) The terminal part containing shoot apex and cotyledon; ii) A sub-apicular prarenchymatous region; and iii) the radicular food storing region bearing absorbing hair.

The first leaf primordium was initiated after the organization of the cotyledonary sheath. It originated in alternating with the cotyledonary sheath and the subsequent leaf primordia development followed formation of the leaves, therefore, these are 2-ranked and distichously arranged as has been reported by earlier workers (Carlson, 1943; Mitra, 1971; Rao, 1967). Hand-in-hand with these changes at the terminal part, the formation of absorbing hairs at the basal part ceases and the first root primordium originated from the upper part of the protocorm at the point where the procambium of stem tip ends blindly.

The root primordium was initiated only after the organization of shoot apex and following initiation of one or two leaf primordia. The first root extended out from a side in the upper part of the protocorm as observed in the present study. A similar location and emergence of the root has been recorded in many other orchids (Bernard, 1909; Carlson, 1943; Mitra, 1971; Veyret, 1974).

A specific location of shoot apex, first leaf primordium and root primordium were noted in the present study. A

similar feature has been recorded in *Dendrobium* (Arekal and Karanth, 1980). This is in contrast to the conclusion made by Rao (1963) who stated that the root primordium does not have a specific location. Further, Rao (1977) opined that the shoot apex is either terminal or lateral in position, however, in the present investigation and in the species studied by Arekal and Karanth (1980), it is consistently noticed that the shoot apex was exogenous and is always terminal in position. The occurrence of a lateral meristem so far recorded is probably based on the observations made on non-median sections. Similarly, the observation of endogenous origin of shoot apex made by Batygina and Vasilyeva (1983) appears to be based on paracotyledonary non-median sections.

As mentioned earlier, the collar-like extension of cells resulted in a scaly cotyledonary sheath encircling epicotylary meristem at the terminal end. Although the previous workers also observed a similar structure, they have interpreted the same in different ways. For example, as a sheathing scale leaf (Mitra, 1971), cotyledonary leaf (Rao, 1967), collar (Prakash and Aow, 1973; Shushan, 1959) and the first leaf (Arditti, 1967; Carlson, 1943). The reason for such varied interpretation on such a structure is undoubtedly due to the fact that none of them ever recognized the cotyledon and epicotyl in the mature orchid embryo. The present interpretation seems from the fact that a single vascular trace is seen in this organ (cotyledonary sheath) in contrast to the leaves in which more number of vascular strands exist. Moreover, the short life followed by senescence of the sheath provides additional evidence in support of this view. Therefore, the previous concept that the first organ to mature during seedling ontogeny is the foliage leaf (Arditti, 1967; Carlson, 1943; Mitra, 1971; Prakash and Aow, 1973; Rao, 1977; Shushan, 1959) is not supported by the present study.

In the present study, like those investigated by Arekal and Karanth (1980) and Batygina and Vasilyeva (1983) the root originated later, as compared to the origin of leaves at the shoot apex. The roots were endogenous as observed in *Arundina bambusifolia* (Mitra, 1971), *Bulbophyllum bufo* (Veyret, 1965), and *Polystachya gearensis* (Veyret, 1974). Nonetheless, there are reports of exogenous origin of roots in orchids (Bernard, 1899; Fuchs and Ziegenspeck, 1926). The first lateral root was initiated at the site proximal to the procambial strand that ends blindly in the protocorm as observed in the present study. Though Carlson (1943), Curtis (1943), Rao (1967), and Shushan (1959) noticed that the root originated from the middle of basal region of the protocorm in proximity with the apical meristem, they did not state that it arises from the presently recognized radicular axis. No organ differentiation is noticed in the central part of the basal storage region either during embryogeny (Swamy, 1949) or during seedling ontogeny. As a consequence, the radicle is completely inadequate. Morel (1971) conducted experiments to study the organogenetic potentialities of protocorm by placing their slices on Knudson's medium. He observed that only cells of the periphery, especially of epidermis regenerated new protocorms while the central core did not show any mitotic activity. This proves that the central core of cells being parenchymatous theoretically totipotent, do not proliferate (Morel, 1971). Similar physiological differences between cells of the periphery and those of the inner core were noticed by Payawal (1977). Such factors, either extrinsic or intrinsic presented at this pole might be responsible for the lack of radicle at this end. Thus, in the family Orchidaceae the primary root is absent, and it involves the initiation of secondary root system. The argument that the first lateral root originates at the base of protocorm is not borne out by the actual facts.

A procambial strand is organized by the central part of the protocorm from cells lying slightly below the shoot apex, a feature also recorded by Batygina and Vasilyeva (1983). In the present study also, 1-2 leaf primordia originated before the organization of procambial strand as also observed by Mitra (1971). The lower limit of the radicular axis is easily discernible as the procambial strand does not extend beyond this region. The cells of the lower region of the radicular axis of protocorm showed signs of degeneration during further stages of seedling ontogeny as noticed in *Dactylorhiza maculata* (Batygina and Vasilyeva, 1983), *Dendrobium lawianum* (Arekal and Karanth, 1980), and *Polystacya geariensis* and *Stanhopea costaricensis* (Veyret, 1974). The consistently observed features that the procambium ends blindly at the lower part of the protocorm has to be considered as an expression of the embryonal radicular axis. The origin of lateral roots occurred nearly at the proximity of the inner limit and this procambial strand- suggesting a nodal plate. Hence, the statement of Meyer (1958) that " the orchid embryo is globose or ovoid and does not differentiate into an elongate axial body possessing apical meristem, node, and other parts; it is merely a block meristem" is not supported by the present study.

During the germination of the orchid seeds, the radicular axis of the embryo enlarged and elongated due to the increase in the size and also stretching of cells at the end. This elongating radicular axis has been designated previously as 'elongating protocorm' (Carlson, 1943) and 'columella' (Mitra, 1971). Since the cotyledon comes

out of the seed coat due to this elongation of the radicular axis, the germination in orchids has to be regarded as epigeal. The arguments such as the 'extremely hypogeal' as in parasitic Angiosperms (Haccius, 1966; Rangaswamy, 1967) or a 'variant of hypogeal type' (Rangaswamy, 1967) amounts to doubting the very existence of cotyledon itself, in these biologically specialised group of monocotyledons, the orchids.The tap root does not exist in the orchid seedlings. The radicular end of the embryo acts as a storage part till the seedling becomes independent, by which stage it disorganizes. The observations of Alvrez and Sagawa (1965a, b) in *Vanda*, that a primary root arises from the meristematic apex and its emergence is associated with necrosis and disappearance of the parenchymatous tissue requires re-investigation. Precocious flowering observed in the present study might be due to the mass effect of plants in the culture flasks. Early flowering has also been reported in *Dendrobium candidum* (Wang *et al*., 1993).

The orchid embryo, while giving rise to a seedling undergoes ontogenetic changes such as formation of cotyledon, epicotylary meristem, plastachronic rhythm, and substitution of tap root by adventitious roots. These are features shared by monocotyledons, in general. The sole deviation in the orchids is the intercalation of what has been generally designated as a protocorm which being called by various names (Batygina and Vasilyeva, 1983). This is a stage invariably met within *in vitro* germination of seeds. Whether such a stage is consistently observed in *in vivo* germination, no adequate data is available. Therefore, it is better we abandon the term protocorm in reference to the orchid embryo till *in vivo* germination of seeds of different species of orchids is thoroughly investigated, to avoid misleading comparisons and consequences. It was Bower (1935) who first gave the term protocorm to a diploid, non-vascular, independent, and tuberoid embryonal phase organized under special environmental conditions in the life cycle of *Lycopodium cernum* of Pteridophytes. Apparently it was not an obligate step in the normal ontogenetic sequence establishing the young vascular root bearing Lycopsida. Whether the protocorm of orchids is an obligate step in *in vivo* ontogeny, similar to *in vitro* ontogeny of the seedling, and how far such a stage stands comparison in structure and function to that of *Lycopodium* needs to be understood.

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