Summary

Germination potential and subsequent differentiation of the seeds of 15 taxa belonging to both epiphytic and terrestrial habits were tested in 4 culture media commonly employed for orchid cultures. Although, seed germination was noticed in all the culture media presently employed, optimum seed germination and subsequent development was observed in Mitra et al. (1976) medium (BM). Seeds of epiphytic taxa germinated with ease as compared to those of the terrestrial ones. In the latter case, browning of the medium probably due to the release of some phenolic compound, in the immediate vicinity of the germinating entities which hampered their growth. This problem could be effectively checked by the addition of 0.2% activated charcoal.

Presently, irrespective of the plant habit, seed germination in vitro seems to be dependent upon the stage of development at the time of inoculation and composition of the culture medium. Immature seeds of Bulbophyllum hirsutum procured between 8-16 weeks after pollination germinated. Failure of seeds procured from dehisced fruits to germinate in vitro indicates the development of some dormant factors in the later stages of seed development.

The germinated seeds responded differently to solid and liquid media of the same nutritional combinations. While,
these thrived better and produced vigorously growing protocorms in solid media, those in liquid combinations showed retardation in their growth. A comparative study of the suitability of the seeds representing different developmental stages both from mature and immature fruits, revealed that those from the mature pods either failed to germinate or germinated sparingly in contrast to the immature seeds from unripened green pods which showed high germinating capabilities.

In all taxa except Cymbidium lowianum where seeds germinated to form rhizomatous structures, more or less similar growth pattern was followed. The first signs of seed germination were apparent in the swelling of the enclosed embryos followed by bursting of seed coats to release enlarged spherules. These further developed into more or less globular and/or pyriform protocorms bearing rhizoids generally at the base or all over depending upon the medium composition. While the seeds of majority of the present taxa readily germinated on BM, their further growth was generally influenced when small quantities of auxins (IAA, NAA or 2,4-D), gibberellin (GA₃), and/or cytokinins (KN or BAP) were supplied exogenously. From amongst the auxins, IAA and/or NAA usually had a benign effect on the germination and/or development of seedlings and their synergistic action when used along with cytokinins, was evident in some cases. On the other hand, 2,4-D generally
inhibited germination and/or differentiation. In the presence of 2,4-D, increased rhizogenesis and/or protocorm callusing was frequently observed. In *Phytochelys rheusa* and *Saccobium calcicolare*, simultaneous protocorm callusing was noticed in the presence of either of the growth supplements with or without the addition of other growth regulator.

Present investigations on the morphogenetic effect of 10 different C-sources (mono-, di-, tri-saccharides and sugar alcohol) at two different concentrations, i.e., 0.5 and 1.0 g/l, on the developing protocorms of *R. retusa* and *Saccobium papillosum* revealed that while in the absence of sucrose, the protocorms failed to differentiate, its presence stimulated differentiation suggesting thereby that an exogenous supply of sucrose is essential for their successful culture. Experiments were designed to find a suitable replacement of sucrose with other sugars for protocorm multiplication and differentiation in *R. retusa* and *S. papillosum*. As indicated in the present studies, the order/preference of different C-sources used in *S. papillosum* is: Xylose > Glucose > Fructose > Sucrose > Mannose > Maltose > Arabinose = Raffinose = Mannitol = Lactose; whereas, the order of their preference for *R. retusa* is: Mannose > Sucrose > Fructose > Xylose > Maltose > Raffinose > Glucose > Mannitol > Lactose > Arabinose.

In sucrose free combinations containing either of the growth retardants coumarin, maleic hydrazide,
2,3,5-triiodobenzoic acid, the protocorms lost chlorophyll and perished within about 4 weeks. Their toxic effect was enhanced with their increased concentration. On the other hand, in the presence of sucrose, these supported abnormal protocorm growth and slow organogenesis. Coumarin in sucrose supplemented media favoured protocorm multiplication and root development at low concentrations. With increase in its concentration, the roots became thickened and shortened.

Maleic hydrazide in the presence of sucrose favoured organogenesis in the protocorms. The roots were thicker with many absorbing hairs. Abnormal protocorm growth was observed at low concentrations of 2,3,5-triiodobenzoic acid and appeared as thin elongated and club shaped structures.

Regeneration potentials of explants procured from leaves (Cymbidium gochristianum, Pulophia hormusjii, Rhynchostylis retusa, and Vanda parviflora); roots (C. gochristianum, Dendrobium nobile, Pachystoma senile, R. retusa, and V. parviflora); tubers (P. senile); rhizome (E. hormusjii) and inflorescence axes (Dendrobium crepidatum, D. pierardii, and Saccolabium calceolare) were variously assessed with a view to achieve mass cloning.

Young leaf segments obtained from plants growing in vivo and/or in vitro were cultured on variously modified BM. The explants from young leaves growing in nature did not show any regeneration and turned necrotic while, those from axenic cultures responded favourably depending upon the
chemical stimulus. Although callusing could not be achieved in either of the nutrient combinations, protocorm-like bodies (plbs) were however, formed.

Root elongation in explants with intact root tips was noticed in C. zamjlanum, Dendrobium moschatum, and P. senile. However, a direct regeneration of shoot buds and/or plbs at the cut ends of explants was achieved in R. retusa, tuber (P. senile) and rhizome (E. hormusijii) explants formed favourable inoculae for mass cloning. Callusing was occasionally seen in 2,4-D containing combinations whereas, protocorm-like bodies (plbs) were regenerated from the cut surfaces of explants in the presence of either of the growth regulators. Regeneration of plbs was enhanced upon addition of yeast extract in P. senile. Direct shoot bud formation was noticed in E. hormusijii in certain selected nutrient combinations. Pseudobulb segments of D. moschatum were also successfully used as explants for cloning purposes.

Present investigations were undertaken to test as to whether the immature floral buds could attain full development in vitro and to what extent morphogenetic pattern could be altered by manipulation of the nutrient medium. Fair amount of success was achieved in the reversal of floral explants to vegetative shoots in D. crepidatum, D. pierardii, and S. celcesolare.

Cytological data from in vitro cultures of the taxa
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under study raised from seeds and/or explants procured from juvenile plant organs made an interesting observation. The regenerants from different explants in C. gammieanum, D. crepidatum, D. moschatum, D. pierardii, E. hormusijii, P. senile, R. retusa, S. calceolare and V. parvilora exhibited stability of their genomes. Similarly, chromosome number stability was observed in the seed cultures of Aerides multiflorum, C. eburneum, C. gammieanum, Coelogyne nitida, and E. hormusijii. A variable number of aneuploid cells were frequently observed in the cultures of D. crepidatum, S. calceolare, Thunia alba, and V. parvilora. On the other hand, both aneuploid and polyploid cells were found scattered with almost equal frequencies amongst the normal diploid cells in taxa like Arundina bambusifolia, D. moschatum, P. senile, and R. retusa. The genomes of A. bambusifolia and P. senile were more prone to chromosomal mutation and variously aneuploid or polyploid cells were observed in the cultural combinations containing auxins (IAA, NAA, 2,4-D) and KN with or without organic growth supplements. These data indicate the importance of nutrients in the origin of deviant cells.

Successful attempts were made to induce polyploidy by the application of colchicine in liquid culture media to the young and actively growing protocorms of C. eburneum, C. gammieanum, and V. parvilora. Most of the cells in their cultures were found to be tetraploid within a period
of about 144 - 168 hours of colchicine treatment. The polyploids thus produced showed phenotypic variations.