IARJSET

ISSN (Online) 2393-8021
ISSN (Print) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology
Vol. 3, Issue 5, May 2016

Asymbiotic Seed Germination in Orchids: Role of Organic Additives

Ashish Gupta

Assistant Professor, Department of Botany, DAV College, Amritsar, India

Abstract: Orchid seeds are unique in being exceedingly small, dust like in appearance, and more or less fusiform in shape; these lack endosperm and have undifferentiated embryos enclosed within transparent seed coats. Their germination in nature is dependent upon a suitable association with a mycorrhizal fungus. Their fungal requirement can, however, be compensated by supply of sugars and other mineral nutrients in vitro, and several orchid species from diverse habits and habitats have successfully responded to asymbiotic germination, much, however, still remains to be learnt about the nutrient requirements of commercially important and/or endangered orchid species, keeping in view of the large size of the orchid family. This paper attempts to review the available literature on various aspects of asymbiotic seed germination in orchids and the role of organic growth additives in promoting seed germination, protocorm development and growth of seedlings.

Keywords: Orchids, asymbiotic seed germination, in vitro culture, organic additives.

I. INTRODUCTION

The Orchidaceae, comprising 30,000–35,000 species belonging to 850 genera, is the largest, highly evolved and diverse family of flowering plants, and accounts for 10% of flowering plants or almost 30% of monocotyledons (Dressler, 1993; Lucksom, 2007). About 70% of orchids are epiphytes and comprise almost two-third of the world's epiphytic flora (Gravendeel et al., 2004). Of the rest, 25% are terrestrial and 5% can be found on various supports (Atwood, 1986). The orchids are well known for their exquisite and long-lasting flowers which have made them doyen among ornamentals. They are characterized by a highly specialized pollination system, small, thin and non-endospermic seeds, obligate requirement of mycorrhizal association during germination, diverse and cosmopolitan habitats, advanced evolutionary trends, extraordinary mechanisms of adaptation and persistence in adverse environmental conditions (Rasmussen, 1995; Arditti, 1992; Phillips et al., 2009). Consequently, they have always fascinated botanists, horticulturists, and evolutionary biologists alike.

The orchids are inherently slow growers; their growth and development are markedly influenced by specialized microclimatic conditions and protective canopy of the floristics in their natural habitats (Vij, 1995). In the recent years, wanton clearance of forests for developmental and agricultural purposes has jeopardized the existence of the natural habitats and populations of a large number of orchids. Unregulated collection of orchids for commercial and herbal purposes has compounded the problem further. As a result, the majority of orchids have become an object of concern to conservationists due to its high sensitivity to alterations in its environment. In situ conservation by preservation and enhancement of dwindling populations of endangered orchid species is very difficult because of the relatively slow growth of orchids and low germination rates which requires symbiotic relationships with mycorrhizal fungi in natural habitats (Rasmussen, 1995). Consequently, in vitro mass propagation techniques are being increasingly utilized for conservation and commercialization of orchid species and hybrids. Ever since the development of a protocol for asymbiotic seed germination of orchids by Knudson (1922), the technique has become an important and favored method for propagation of a variety of terrestrial and epiphytic orchid species (Yamazaki and Miyoshi 2006; Stewart and Kane 2006).

II. ORCHID SEEDS

The structure and size of the seed are among the most striking characteristics of orchids. Orchid seeds are very small (0.05-6.0 mm in length and 0.01-0.9 mm in diameter), extremely light (0.31-24 □ g), and produced in large number (50-4,000,000 per capsule) (Arditti and Ghani, 2000). The seed, which may at times be apomictic (Stoutamire, 1964), has been compared to fern spores (Went, 1949) and consists of a small spherical embryo suspended within a membranous, often transparent, but at times pigmented, seed coat (Arditti, 1967). In most orchids, the embryo is undifferentiated and the endosperm development is suppressed (Cocucci and Jensen, 1969). While, the mature embryo of Epipogium aphyllum always comprises 8 cells, the number of cells in the mature embryo, in a majority of species, has been reported to range from 29 (Calypso bulbosa; Harvais, 1974) to ca. 200 (Dactylorhiza majalis; Rasmussen, 1990). Bletilla striata, considered to possess one of the most highly developed embryos, comprises about 734 cells (Rasmussen, 1995).
The orchid seeds at maturity contain lipidaceous food reserves, which occur as discrete inclusions within the cells of the embryo (Poddubnaya-Arnoldi and Zinger, 1961). Analysis of Cymbidium seeds showed that they contain 32% lipids, 1% sugars, and no starch (Knudson, 1929), although starch is reported in the embryos of other orchid

species (Arditti and Ernst, 1981). Because of the limited food reserves in the minute seeds, orchids in their natural habitats go through a heterotrophic phase in which the developing plants rely on a balanced relationship with fungal endophytes for nutrition, and this reliance begins at the onset of germination (Smreciu and Currah, 1989). Since the uninfected orchid seedlings are unable to grow on polysaccharides and attempts to germinate seeds asymbiotically in the absence of simple sugars are invariably unsuccessful, even with the addition of vitamins, hormones, or other substances (Arditti and Ernst, 1981), the need for fungal infection of orchid seeds appears to be due to an impaired ability of the seeds to metabolize polysaccharides and lipids (Manning and van Staden, 1987). The fungus is believed to provide the necessary germination stimulus by aiding carbohydrate, nitrogen, mineral, and vitamin transport during germination (c.f. Arditti, 1967).

III. IMMATURE SEED GERMINATION

Ever since Knudson (1922) demonstrated that the fungal requirement of orchid seeds can be successfully bypassed in vitro using a relatively simple culture medium containing sucrose, asymbiotic seed germination has emerged as an important procedure for propagating a large number of orchid species and hybrids representing diverse habits and habitats (Arditti et al., 1982; Miyoshi and Mii, 1995, 1998; Pathak et al., 2001; Piri et al., 2012). The orchid seeds are also capable of germination, in vitro, prior to reaching maturity, and their culture often referred to as "ovule/embryo/green-pod" culture (Sagawa, 1963) has added new dimensions to conservation and commercialization of orchids.

The technique has been positively tested in a large number of taxa (Arditti et al., 1982; Vij and Pathak, 1988; Yam and Weatherhead, 1988; Shiau et al., 2002). The technique involves an easy procedure of sterilization, ensures better frequency of germination, reduces the time lapse between pollination and sowing of seeds, and helps in i) production of virus-free seedlings, ii) propagation of rare and endangered species, and iii) recovering progenies of desired matings (Vij et al., 2000). Its additional utility in exploiting the polyembryonate potential of orchid seeds and cloning their apomictic (obligate) genotypes has also been highlighted in several ground orchids which often tend to bypass sexuality in favour of apomixis (Vij, 1995).

The better germination potential of immature seeds has been attributed to their distended testa cells and metabolically awakened embryos besides lack of dormancy and/or inhibitory factors (Linden, 1980; Yam and Weatherhead, 1988). The desiccation that takes place in the intact maturing fruit is interrupted when ovules are excised and transferred to a culture medium with low osmolarity. Since declining water potential is probably a regulatory factor for protein accumulation in ripening seeds, their rehydration during germination in vitro generates a developmental change from protein storage to protein mobilization (Kermode, 1990). However, as all the seeds/embryos are used in a single sowing in the "green-pod" culture technique, the importance of a proper stage at which the capsule has to be harvested in a particular taxon assumes great significance. The immature seeds are often excised while the embryo cells are still fully hydrated and mitotically active. Bhojwani and Razdan (1996) reported that the nutritional requirements of the young embryos are complex as compared to the mature ones. According to Arditti et al. (1982), the seeds collected from capsules after about half of the time they take to mature show a better germination response. Based on a comparative analysis in 47 species and hybrids, Nagashima (1989) hinted at a strong correlation between periods from pollination to fertilization and from pollination to the completion of embryogenesis, and also between the periods from pollination to fertilization and from pollination to the stage of maximum germinability.

IV. MATURE SEED GERMINATION

The mature seeds, on the other hand, often germinate with difficulty due to change in the quality of their food reserves. Harvais (1974) reported that food reserves comprise starch in immature, and lipids in the mature seeds of Corallorhiza maculata and suggested that conversion of starch and other simpler carbohydrates into lipids during seed maturation may be a common feature of orchids. The inability of mature seeds to germinate with ease has also been attributed to the lack of an appropriate metabolic machinery (glyoxysomes) capable of utilizing their own lipidaceous reserves (Harrison, 1977); accumulation of germination inhibitors in the seed coat; onset of dormancy in the mature seed; and loss of viability (c.f. De Pauw and Remphrey, 1993). Since dormancy of mature seeds in orchids has been successfully broken by treating these with cold temperature (Harvais, 1974) or sucrose (Weatherhead et al., 1986), it appears that dormancy rather than the non-viability is responsible for poor response of the mature seeds in vitro.

In Dactylorhiza maculata, the abscisic acid contents which are 15 times more in the mature seeds as compared to the immature ones, may be responsible for seed dormancy (Van der Kinderen, 1987). Raghavan (1976) recorded changes in the enzyme complements at different stages of seed maturation in orchids, but the critical stage at which they acquire dormancy, is yet to be identified. Moreover, as the dormant (mature) and metabolically active (immature) seeds are morphologically more or less indistinct, Mitra (1986) stressed the importance of information on histochemical and biochemical features for selecting the right type of immature ovules (seeds).

The epiphytic species germinate better than the terrestrial ones due probably to their simpler nutritional requirements (Arditti et al., 1982), and the present species were no exception. The stringent nutritional requirements of the terrestrials for germination in vitro have been attributed to their greater mycorrhizal needs (Stoutamire, 1974). In this connection, it is worthwhile to mention that in vitro requirements of seeds/embryos are greatly influenced by the level of their maturity and genetic and ecological amplitudes of the species (Anderson, 1991).

V. EFFECT OF ORGANIC ADDITIVES

Growth and morphogenesis of plant tissues can be promoted by the addition of various organic supplements and plant extracts (Fonnesbech 1972). A large and bewildering number of organic additives, unwanted or untried in other plants, are routinely employed to enrich the culture media for orchid seed germination. Some such common as well as uncommon additives are apple juice, banana homogenate, beef extract, casein hydrolysate, coconut water, extract of silkworm pupae, fish extract, honey, peptone, potato extract, tomato juice, and yeast extract, although they have undefined mixture of organic nutrients and growth factors (Islam et al. 2003; Murdad et al. 2010).

Peptone

A water soluble protein hydrolysate with high amino acid content, Peptone (P) supported growth of P. spicerianum cultures (Flamee, 1978). A perusal of literature reveals that P was obligatory for germination in Spiranthes cernua (Stoutamire, 1974) and for inducing organogenesis in Goodyera biflora (Pathak et al., 1992). It improved germination [Dactylorhiza (Linden, 1980)], supported seedling growth [Paphiopedilum, Phaius, Vanda (Curtis, 1947), Geodorum densiflorum (Roy and Banerjee, 2001)], and induced protocorm multiplication [Cymbidium macrorhizon (Vij and Pathak, 1988)]. Growth promotion of protocorms by P has been reported for orchid species like Spathoglottis plicata (Curtis, 1947), Epidendrum ibaguense (Hossain, 2008), and Calopogon tuberosus (Kauth et al. 2006). However, P impaired germination in Dactylorhiza maculata (Van Waes and Debergh, 1986) and Vanda tessellata (Roy and Banerjee, 2002), and proved ineffective in Calanthe discolor (Kano, 1965) cultures. All these data suggest that efficacy of P varies with the species and the developmental stage of the germinating entities. According to Arditti at al. (1982), the discordant effect may also be attributed to the source and batch of P supply.

Coconut Water

Coconut water (CW) is the colorless liquid endosperm of green coconuts (Cocos nucifera L.), which contain soluble sugars as a natural source of carbon, amino acids, phenols, fiber and vitamins, moreover, it also contains diphenyl urea which functions as cytokinin that can enhance the explant growth and regeneration by inducing cell division (Gnasekaran et al., 2010; Texeira da Silva et al., 2006). For years, simple supplementation of nutrient media with CW has remained a standard procedure to obtain satisfactory growth and organ differentiation in orchid cultures. CW induces division of the otherwise non-dividing cells (George and Sherrington, 1984) and mass multiplication of protocorms (Sagawa and Kunisaki, 1984) in orchids. The concentration of CW employed for this purpose ranges from 10-30% (Sagawa and Kunisaki, 1984). The growth promotory effect of CW has been attributed to its PGR content, and the most important PGRs are the cytokinins (Letham, 1974; van Staden and Drewes, 1975), whereas auxin and gibberellin-like constituents have also been reported (Dix and van Staden, 1982). Huang and Hu (2001) reported that 5% CW, banana homogenate or potato homogenate in Harvais medium could accelerate the seed germination of Cypripedium flavum. Similar results have been reported in different plant species. For instance, the fresh and dry biomass of Anoectochilus formosanus increased significantly when 50 ml/L CW and 0.5 g/L activated charcoal were added during bioreactor culture (Yoon et al., 2007). Hyponex medium supplemented with 50 ml/L CW enhanced fresh and dry biomass, number of roots, leaf area as well as development of healthy plantlets of Calanthe hybrids (Baque et al., 2011). Similarly, 10% CW promoted the proliferation of protocorm-like bodies of Dendrobium Alya Pink (Nambiar et al., 2012).

Recently, Huh et al. (2016) analyzed the constituents of CW and reported that it contained around 2.5% sugars and sucrose was the main sugar. CW also contains variable inorganic ions such as potassium, phosphorus, calcium, magnesium, iron and manganese. Water-soluble vitamins such as thiamin (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), myo-inositol and ascorbic acid (C), which were recommended for orchid growth, were also detected in CW. Coconut water also contained organic acids including citric acid, malic acid and succinic acid. Phytohormones such as Indoleacetic acid, trans-Zeatin riboside and Abscisic acid were also detected in CW.

Banana Homogenate

Graefflinger (1950; cited in Ernst et al., 1970) first proposed the use of banana fruit powder for the germination of orchids. Withner (1953) observed beneficial role of mashed banana or banana homogenate (BH) in the germination of Paphiopedilum seeds. Ernst (1982) recorded a growth promotory effect of BH on Paphiopedilum seedlings. An analysis of banana fruit pulp revealed that it contains different carbohydrates, minerals, amino acids, fatty acids, niacin, vitamins, cellulose, polyols, and sterols (Tamura, 1970). The stimulatory effect of BH on seed germination and seedling development has also been observed in Anoectochilus formosanus (Shiau et al., 2002), Cattleya (Islam et al., 2000), Hetaeria cristata (Yam and Weatherhead, 1990), and Phalaenopsis (Ernst, 1986). In studies with Paphiopedilum ciliolare, Pierik et al. (1988) reported that the effect of BH was inhibitory during seed germination and beneficial only for further development of seedlings.

Yeast Extract

Yeast Extract (YE) is also an important source of organic nitrogen (amino acids) and has been effectively used in germination and proliferation in many orchid species (Mitra, 1986).

Studies conducted in terrestrial orchid seeds and protocorms revealed more efficient utilization of amino acids by young protocorms (Curtis 1947; Curtis and Spoerl 1948; Malmgren 1996). Greater preference for nitrogen from amino acids rather than from ammonium or nitrate salts has also been favored for some epiphytic orchids (Nadarajan et al. 2011).

Casein Hydrolysate

Casein hydrolysate (CH) is an amino acid complex and has been widely used in orchid seed germination media. Beneficial role of CH in seed germination and seedling growth has been demonstrated in Dactylorhiza purpurella (Harvais, 1972), Aerides multiflora, Rhynchostylis retusa, Saccolabium papillosum, Vanda testacea (Vij et al., 1981), and Bletia urbana (Rubluo et al., 1989). Decruse et al. (2013) have also reported enhanced growth of protocorms of Eulophia cullenii in medium supplemented with CH. However, CH was reported to impair germination in Herminium lanceum and seedling development in Coelogyne barbata (Chand, 1991). Growth promotory effect of CH was not pronounced in Dactylorhiza maculata (Van Waes and Debergh, 1986) and Vanda cv. Miss Joaquim (Rao and Avadhani, 1964) cultures. The variable effect of CH during germination may be attributed to its composition, which varies with extent of hydrolysis of the nitrogenous substances present in the casein (Arditti et al., 1982).

VI. CONCLUSION

Asymbiotic seed germination provides an efficient way for mass propagation of orchids. Development of efficient seed germination and acclimatization protocols that focus on propagating orchid seedlings for reintroduction in their natural habitats will help in the conservation of endangered or threatened orchid species. Addition of organic additives to orchid seed germination medium promotes seed germination, accelerates protocorm formation and produces vigorous seedling in a majority of the reports in literature. These organic additives provide a natural source of carbohydrates, inorganic ions, amino acids, vitamins and phytohormones, and help in orchid propagation by promoting growth and morphogenesis in asymbiotic seed cultures.

REFERENCES

[1]. Anderson, A. B. 1991. Symbiotic and asymbiotic germination and growth of Spiranthes magnicamporum (Orchidaceae). Lindleyana, 6: 183-186.
[2]. Arditti, J. 1967. Factors affecting the germination of orchids. Bot. Rev., 33: 1-97.
[3]. Arditti, J. 1992. Fundamentals of Orchid Biology. John Wiley & Sons, New York.
[4]. Arditti, J. and R. Ernst. 1981. Metabolism of germinating seeds of epiphytic orchids: an explanation for the need for fungal symbiosis. In: Proc. 10th World Orchid Conference (eds. J. Stewart and C. N. Van der Merwe) pp. 263-267. L. Backhouse (Pty) Ltd., Peitermaritzburg.
[5]. Arditti, J. and A. K. A. Ghani. 2000. Numerical and physical properties of orchid seeds and their biological implications. New Phytol., 145: 367-421.
[6]. Arditti, J., M. A. Clements, G. Fast, G. Hadley, G. Nishimura, and R. Ernst. 1982. Orchid seed germination and seedling culture- A manual. In: Orchid Biology- Reviews and Perspectives, Vol. II (ed. J. Arditti) pp. 243-370. Cornell University Press, Ithaca, New York.
[7]. Atwood, J. T. 1986. The size of orchidaceae and the systematic distribution of epiphytic orchids. Selbayana, 9: 171-186.
[8]. Baque MA, Shin YK, Elshmari T, Lee EJ, Paek KY (2011) Effect of light quality, sucrose and coconut water concentration on the micropropagation of Calanthe hybrids ('Bukduseong' x 'Hyesung') Aust J Crop Sci 5:1247-1254
[9]. Bhojwani, S. S. and M. K. Razdan. 1996. Plant Tissue Culture: Theory and Practice, A Revised Edition. Elsevier, Amsterdam.
[10]. Chand, K. 1991. Green Pod Culture and Regeneration Potential of Some Indian Orchids: A Study In Vitro. Ph.D. Thesis, Panjab University, Chandigarh.
[11]. Cocucci, A. and W. A. Jensen. 1969. Orchid embryology: Megagametophyte of Epidendrum scutella following fertilization. Am. J. Bot., 56: 629-640.
[12]. Curtis, J. T. 1947. Studies on the nitrogen nutrition of orchid embryos. I. Complex nitrogen sources. Am. Orchid Soc. Bull., 16: 654-660.
[13]. Curtis, J. T. and E. Spoerl. 1948. Studies on the nitrogen nutrition of orchid embryos. Am. Orchid Soc. Bull., 17: 111-114.
[14]. De Pauw, M. A. and W. R. Remphrey. 1993. In vitro germination of three Cypripedium species in relation to time of seed collection, media, and cold treatment. Can. J. Bot., 71: 879-885.
[15]. Decruse SW, N. Reny, S. Shylajakumari, and P. N. Krishnan. 2013. In vitro propagation and field establishment of Eulophia cullenii (Wight) Bl., a critically endangered orchid of Western Ghats, India through culture of seeds and axenic seedling-derived rhizomes. In Vitro Cell. Dev. Biol.-Plant, 49:520–528.
[16]. Dix, L. and J. van Staden. 1982. Auxin and gibberellin-like substances in coconut milk and malt extract. Plant Cell Tiss. Org. Cult., 1: 239-246.
[17]. Dressler, R. L. 1993. Phylogeny and Classification of the Orchid Family. Dioscorides Press, Portland.
[18]. Ernst, R. 1982. Paphiopedilum. In: Orchid Biology- Reviews and Perspectives, Vol. II (ed. J. Arditti) pp. 350-353. Cornell University Press, Ithaca, New York.
[19]. Ernst, R. 1986. Seed and clonal propagation of Phalaenopsis. In: Proc. Fifth ASEAN Orchid Congress Seminar (1984) (ed. A. N. Rao) pp. 31-41. Parks & Recreation Department, Ministry of National Development, Singapore.
[20]. Ernst, R., J. Arditti, and P. L. Healey. 1970. The nutrition of orchid seedlings. Am. Orchid Soc. Bull., 39: 599-605, 691-700.
[21]. Flamee, M. 1978. Influence of selected media and supplements on the germination and growth of Paphiopedilum seedlings. Am. Orchid Soc. Bull., 47: 419-423.
[22]. Fonnesbech M 1972. Organic nutrients in the media for propagation of Cymbidium in vitro. Physiologia Plantarum, 27:360-364.
[23]. Gnasekaran P, Xavier R, Uma Rani S, Sreeramanan S (2010) A study on the use of organic additives on the protocorm-like bodies (PLBs) growth of Phalaenopsis violacea orchid. J Phytol 2:29-33
[24]. George, E. F. and P. D. Sherrington. 1984. Plant Propagation by Tissue Culture: Handbook and Directory of Commercial Laboratories. Exegetics, Hants, U.K.
[25]. Gravendeel, B., Smithson, A., Slik, F. J., and Schuiteman, A. 2004. Epiphytism and pollinator specialization: drivers for orchid diversity? Philos. Trans. R. Soc. Lond. B. Biol. Sci. 359: 1523–1535.
[26]. Harrison, C. R. 1977. Ultrastructural and histochemical changes during the germination of Cattleya aurantiaca (Orchidaceae). Bot. Gaz., 138(1): 41-45.
[27]. Harvais, G. 1972. The development and growth requirements of Dactylorhiza purpurella in asymbiotic culture. Can. J. Bot., 50: 1223-1229.
[28]. Harvais, G. 1974. Notes on the native orchids of Thunder Bay, their endophytes and symbionts. Can. J. Bot., 52: 451-460.
[29]. Huh YS, JK Lee, SY Nam, KY Paek and GU Suh. 2016. Improvement of asymbiotic seed germination and seedling development of Cypripedium macranthos Sw. with organic additives. J Plant Biotechnol, 43:138–145.
[30]. Islam, M. O., S. Matsui, and S. Ichihashi. 2000. Effects of complex organic additives on seed germination and carotenoid content in Cattleya seedlings. Lindleyana, 15(2): 81-88.
[31]. Islam MO, Rahman ARMM, Matsui S, Prodhan AKMA (2003) Effects of complex organic extracts on callus growth and PLB regeneration through embryogenesis in the Doritaenopsis orchid. Jpn Agric Res Quart 4:229-235
[32]. Kano, K. 1965. Studies on the media for orchid seed germination. Mem. Fac. Agric. Kagawa Univ., 20: 1-68.
[33]. Kauth PJ, Vendrame WA, Kane ME (2006) In vitro seed culture and seedling development of Calopogon tuberosus. Plant Cell Tiss Organ Cult 85:91–102
[34]. Kermode, A. R. 1990. Regulatory mechanisms involved in the transition from seed development to germination. Critical Rev. Plant Sci., 9: 155-195.
[35]. Knudson, L. 1922. Non-symbiotic germination of orchid seeds. Bot. Gaz., 73: 1-25.

[36]. Knudson, L. 1929. Physiological investigations on orchid germination. In: Proc. International Congress Plant Science, Vol. 2. pp. 1183-1190.
[37]. Letham, D. S. 1974. Regulators of cell division in plant tissues XX. The cytokinins of coconut milk. Physiol. Plant., 32: 66-70.
[38]. Linden, B. 1980. Aseptic germination of seeds of Northern terrestrial orchids. Ann. Bot. Fennici, 17: 174-182.
[39]. Lucksom, S. Z. 2007. The orchids of Sikkim and North-east Himalaya. Author Publishers and Distributors, Gangtok, East Sikkim, Assam, India.
[40]. Malmgren S (1996) Orchid propagation: theory and practice. In: Allen C (ed) North American native orchids: propagation and production. North American Native Terrestrial Orchid Conference, Germantown, Maryland, USA, pp 63–71.
[41]. Manning, J. C. and J. van Staden. 1987. The development and mobilisation of seed reserves in some African orchids. Aust. J. Bot., 35: 343-353.
[42]. Mitra, G. C. 1986. In vitro culture of orchid seeds: obtaining seedlings. In: Biology, Conservation, and Culture of Orchids (ed. S. P. Vij) pp. 401-412. Affiliated East-West Press, New Delhi.
[43]. Miyoshi, K. and M. Mii. 1995. Phytohormone pre-treatment for the enhancement of seed germination and protocorm formation by the terrestrial orchid, Calanthe discolor (Orchidaceae), in asymbiotic culture. Scientia Hortic., 63: 263-267.
[44]. Miyoshi, K. and M. Mii. 1998. Stimulatory effects of sodium and calcium hypochlorite, pre-chilling and cytokinins on the germination of Cypripedium macranthos seed in vitro. Physiol. Plant., 102: 481-486.
[45]. Murdad R. Latip MA, Aziz ZA, Ripin R (2010) Effects of carbon source and potato homogenate on in vitro growth and development of Sabah's endangered orchid: Phalaenopsis gigantea. Asia-Pac J Mol Biol 18:199-202
[46]. Nadarajan J, Wood S, Marks TR, Seaton PT, Pritchard HW (2011) Nutritional requirements for in vitro seed germination of 12 terrestrial, lithophytic and epiphytic orchids. J Trop Forest Sci 23:204–212
[47]. Nagashima, T. 1989. Embryogenesis, seed formation and immature seed germination in vitro in Ponerorchis graminifolia Reichb.f. J. Japan. Soc. Hort. Sci., 58: 187-194.
[48]. Nambiar N, Tee CS, Maziah M (2012) Effects of organic additives and different carbohydrate sources on proliferation of protocorm like bodies in Dendrobium Alya Pink. Plant Omics J 5:10-18
[49]. Pathak, P., K. C. Mahant, and Ashish Gupta. 2001. In vitro propagation as an aid to conservation and commercialization of Indian orchids: Seed culture. In: Orchids: Science and Commerce (eds. P. Pathak, R. N. Sehgal, N. Shekhar, M. Sharma, and A. Sood) pp. 319-362. Bishen Singh Mahendra Pal Singh, Dehra Dun.
[50]. Pathak, P., S. P. Vij, and K. C. Mahant. 1992. Ovule culture in Goodyera biflora: A study in vitro. J. Orchid Soc. India, 6: 49-53.
[51]. Phillips, R. D., Faast, R., Bower, C. C., Brown, G. R., and Peakall, R. 2009. Implications of pollination by food and sexual deception for pollinator specificity, fruit set, population genetics and conservation of Caladenia (Orchidaceae). Aus. J. Bot. 57: 287–306.
[52]. Pierik, R. L. M., P. A. Sprenkels, B. Van der Harst, and Q. G. Van der Meys. 1988. Seed germination and further development of Paphiopedilum ciliolare Pfitz. in vitro. Scientia Hortic., 34: 139-153.
[53]. Piri, H., P. Pathak, and R. K.Bhanwra. 2012. Asymbiotic germination of immature embryos of a medicinally important epiphytic orchid Acampe papillosa (Lindl.) Lindl. African Journal of Biotechnology Vol. 12(2), pp. 162-167.
[54]. Poddubnaya-Arnoldi, V. A. and N. V. Zinger. 1961. Application of histochemical technique to study of embryonic processes in some orchids. Recent Advances in Botany (Univ. Toronto Press), Section 8: 711-714.
[55]. Raghavan, V. 1976. Experimental Embryogenesis in Vascular Plants. Academic Press, New York.
[56]. Rao, A. N. and P. N. Avadhani. 1964. Some aspects of in vitro culture of Vanda seeds. In: Proc. 4th World Orchid Conference (ed. B. C. Yeoh) pp. 194-202. Straits Time Press Ltd., Singapore.
[57]. Rasmussen, H. N. 1990. Cell differentiation and mycorrhizal infection in Dactylorhiza majalis (Rchb.f.) Hunt and Summerh. (Orchidaceae) during germination in vitro. New Phytol., 116: 137-147.
[58]. Rasmussen, H. N. 1995. Terrestrial Orchids: From Seed to Mycotrophic Plant. Cambridge University Press, Cambridge.
[59]. Roy, J. and N. Banerjee. 2001. Cultural requirements for in vitro seed germination, protocorm growth and seedling development of Geodorum densiflorum (Lam.) Schltr. Indian J. Exp. Biol., 39: 1041-1047.
[60]. Roy, J. and N. Banerjee. 2002. Optimization of in vitro seed germination, protocorm growth and seedling proliferation of Vanda tessellata (Roxb.) Hook. ex G. Don. Phytomorphology, 52: 167-178.
[61]. Rubluo, A., V. Chavez, and A. Martinez. 1989. In vitro seed germination and re-introduction of Bletia urbana (Orchidaceae) in its natural habitat. Lindleyana, 4(2): 68-73.
[62]. Sagawa, Y. 1963. Green pod cultures. Florida Orchidist, 6: 296-297.
[63]. Sagawa, Y. and J. T. Kunisaki. 1984. Clonal propagation of orchids. In: Cell Culture and Somatic Cell Genetics of Plants, Vol. I (ed. I. K. Vasil) pp. 61-67. Academic Press, Orlando, Florida.
[64]. Shiau, Y.-J., Sagare, A. P., U.-C. Chen, S.-R. Yang, and H.-S. Tsay. 2002. Conservation of Anoectochilus formosanus Hayata by artificial cross-pollination and in vitro culture of seeds. Bot. Bull. Acad. Sin., 43: 123-130.
[65]. Smreciu, E. A. and R. S. Currah. 1989. Symbiotic germination of seeds of terrestrial orchids of North America and Europe. Lindleyana, 4: 6-15.
[66]. Stewart SL, Kane ME (2006) Asymbiotic seed germination and in vitro seedling development of Habenaria macroceratitis (Orchidaceae), a rare Florida terrestrial orchid. Plant Cell Tiss Org Cult 86:147–158
[67]. Stoutamire, W. P. 1964. Seeds and seedlings of native orchids. Michigan Bot., 3: 107-119.
[68]. Stoutamire, W. P. 1974. Terrestrial orchid seedlings. In: The Orchids- Scientific Studies (ed. C. L. Withner) pp. 101-128. Wiley-Interscience, New York.
[69]. Tamura, S. 1970. Amino acid composition of food in Japan. Japan Agric. Res. Quart., 5: 56-60.
[70]. Texeira da Silva JA, Chan MT, Chai ML, Tanaka M (2006) Priming abiotic factors for optimal hybrid Cymbidium (Orchidaceae) PLB and callus induction, plantlet formation, and their subsequent cytogenetic stability analysis. Sci Hortic 109:368-378
[71]. Van der Kinderen, G. 1987. Abscisic acid in terrestrial orchid seeds: A possible impact on their germination. Lindleyana, 2: 84-87.
[72]. van Staden, J. and S. E. Drewes. 1975. Identification of zeatin and zeatin-riboside in coconut milk. Physiol. Plant., 34: 106-109.
[73]. Van Waes, J. M. and P. C. Debergh. 1986. In vitro germination of some Western European orchids. Physiol. Plant., 67: 253-261.
[74]. Vij, S. P. 1995. Genetic resources of orchids. In: Advances in Horticulture Vol. 12- Ornamental Plants I (eds. K. L. Chadha and S. K. Bhattacharjee) pp. 153-181. Malhotra Publishing House, New Delhi.
[75]. Vij, S. P. and P. Pathak. 1988. Asymbiotic germination of saprophytic orchid, Cymbidium macrorhizon: A study in vitro. J. Orchid Soc. India, 2: 25-32.
[76]. Vij, S. P., A. Kher, and Ashish Gupta. 2000. Orchid Micropropagation. In: Biotechnology in Horticultural and Plantation Crops (eds. K. L. Chadha, P. N. Ravindran, and L. Sahijram) pp. 598-641. Malhotra Publishing House, New Delhi.
[77]. Vij, S. P., A. Sood and K. K. Plaha. 1981. In vitro seed germination of some epiphytic orchids. In: Contemporary Trends in Plant Sciences (ed. S. C. Verma) pp. 473-481. Kalyani Publishers, New Delhi.
[78]. Weatherhead, M. A., S. Y. Zee, and G. Barretto. 1986. Some observations on the early stages of development of Eulophia yushuiana. Mem. Hong Kong Natural History Soc., 17: 85-90.
[79]. Went, F. W. 1949. The plants of Krakatoa. In: Plant Life - A Scientific American Book. pp. 137-145. Simon and Schuster, New York.
[80]. Withner, C. L. 1953. Germination of "Cyps". Orchid J., 2: 473-477.
[81]. Yam, T. W. and M. A. Weatherhead. 1988. Germination and seedling development of some Hong Kong orchids. I. Lindleyana, 3: 156-160.
[82]. Yam, T. W. and M. A. Weatherhead. 1990. Early growth of seedlings of Hetaeria cristata and plantlet initiation from rhizome nodes. Lindleyana, 5: 199-203.
[83]. Yamazaki J, Miyoshi K (2006) In vitro asymbiotic germination of immature seed and formation of protocorm by Cephalanthera falcata (Orchidaceae). Ann Bot 98:1197–1206.
[84]. Yoon YJ, Murthy HN, Hahn EJ, Paek KY (2007) Biomass production of Anoectichilus formosanus Hayata in a bioreactor system. J Plant Biol 50:573-576.