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Orchids: Advances in Tissue Culture, Genetics, Phytochemistry and Transgenic Biotechnology

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ABSTRACT

Orchids include some of the world's most important floricultural (cut-flower) and ornamental (pot and garden) plants, some of which have pharmacological interest. Even though seed propagation has been recorded since the early 19^{th} century, their micropropagation and tissue culture dominated orchid biotechnology until the 1990's, while transformation and molecular technologies are now being more intensely focused. This review highlights literature until about 2005 that pertains to ornamental orchid *in vitro* cell, tissue and organ culture, micropropagation, genetics and transformation, and takes an in depth analysis at how each of these disciplines has influenced the use of biotechnology in the improvement and preservation of orchids around the world allowing orchid research to take a new direction in recent years. Closely related to this is the use of phytochemicals and secondary metabolites from orchids, which serve important medical and industrial purposes. Using biotechnology, these phytochemicals can be further explored and manipulated *in vitro*.

Keywords: orchid, cryopreservation, genetic transformation, medicinal compounds, postharvest technology, protocorm-like body, secondary metabolites, tissue culture

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INTRODUCTION

Orchids, the doyens among ornamentals, are one of the most important global cut flower and pot plants, and their sheer beauty has enchanted and fascinated people since early times. The Orchidaceae, one of the largest families of flowering plants, has more than 800 genera and 25-35,000 species (Arditti 1992). For Greeks, orchids were a symbol of virility for Chinese, and as far back as Confucious, orchids were termed "plants of the King's fragrance" (Dressler 1981; Hew 2001). Orchid cultivation is one of the most economically significant global nursery industries constituting a multi-billion dollar industry (Hew 1989; Goh and Kavaljian 1989; Alam *et al*. 2002); now with the advent of biotechnology, most desirable and important plants can be cloned using tissue culture as occurs with *Dendrobium*, which accounts for about 80% of the total micropropagated tropical orchids, usually by protocorms (Griesbach 2003; Saiprasad *et al*. 2004). *Cymbidiums* are the most important orchids in commerce, most of which are hybrids derived from the original 44 species (Obara-Okeyo and Kako 1998). Monopodial orchids such as *Vanda*, *Ascocentrum*, *Rhynchostylis*, *Aranda*, *Renanthera* and *Neofinetia*, and intergeneric hybrids of these genera are widely cultivated, and whose select cultivars are bred by tissue culture to overcome the low propagation rate by conventional methods.

The world export/import trade of orchid cut flowers and orchid plants exceeded $150 million in 2000, $128 comprising the former (Laws 2002). Asia dominates world trade, with Thailand exporting $50 million in cut orchids, followed by Singapore, Malaysia and New Zealand (Hew 1989; Goh and Kavaljian 1989).

In the preface of their book, Arditti and Ernst (1993) made a comment which still remains pertinent to orchid research today: "photocopy machines… and micropropagation of orchids through tissue culture appeared on the scene almost simultaneously, and the world has not been the same since." With the rapid advance of plant biotechnology, and the advent of numerous molecular techniques, this review aims to cover the rapid advances in orchid tissue culture,

Received: 1 January, 2012. Accepted: 10 January, 2013.

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genetic engineering and postharvest technology that have been made over the past decade, while also giving a historical account of the advances made in tissue culture since the 19th century. Until 2005, only a single review published in the main-stream (Sheehan 1984) described advances in orchid botany, propagation and physiology while Arditti (1984) looks at a historical perspective of orchid hybridization, seed germination and tissue culture. Hew and Clifford (1993) gave a very superficial and fleeting description of the use of plant growth regulators (PGRs) in orchid tissue culture and the cut-flower industry. A much more recent review describes the latest advances in orchid biotechnology and is meant to be the supplement to this review (Hossain *et al*. 2013).

REGENERATION AND DEVELOPMENT

The need for mass propagation of selected elite orchid genotypes and the fact that many economically important orchids develop protocorms slowly in culture led to the development of several *in vitro* methods including culture of flower stalk tips and inflorescences, shoot and stem explants, especially thin cross sections (Prakash *et al*. 1996), root tips, axillary and apical/meristematic buds, and flower stalk buds (**Table 1**). The need to conserve, improve, (cryo)preserve and study the physiology of orchids has also been an impetus for their tissue culture studies (Arditti and Ernst 1993). Plantlets are obtained from these explants through protocorm-like bodies (PLBs) from intermediate callus (Philip and Nainar 1986; Begum *et al*. 1994a) proliferated on media supplemented with additives such as banana homogenate, coconut milk or tomato juice, although orchid callus in general exhibits a necrotic tendency and slow growth (Kerbauy 1984a; Philip and Nainar 1986; Kerbauy 1991; Colli and Kerbauy 1993; Begum *et al*. 1994a). The term PLB, first coined by Morel (1960) describes structures that resemble protocorms but are formed by tissue explants and/or callus *in vitro*. *Cymbidium* PLBs themselves are regenerated from epidermal cells (Tanaka *et al*. 1975; Kim and Kako 1984; Lin 1987), but Begum *et al*. (1994a, 1994b) reported the induction of callus and PLBs from the inner, parenchymatous tissue of PLBs. Zhou (1995) claimed that hyperhydric *Doritaenopsis* PLBs had a greater capacity of differentiation than normal PLBs, but through the addition of potato juice to the medium, normal PLBs could be recovered. Whereas Fukai *et al*. (2002) showed that PLB epidermal tissue had only 2C and 4C peaks, Fujii *et al*. (1999a, 1999b) claimed that most cells in the outer tissue of *Cymbidium* PLBs had a 2C DNA content, but following an NAA treatment, that nuclear DNA contents increased up to 16C (i.e. higher nuclear polyploidy), correspondent to increased cell size (Nagl 1972). Lactopropionic orcein stain has been shown to be suitable for mitotic chromosome counts in orchids (Latha 2002). PLB cell division occurred from the outer cell layers inward, and the frequency of cell division gradually increased with culture time in BA or PGR-free media; frequency of anticlinal cell division was dominant in the outer three cell layers, whereas periclinal cell division was higher in the inner cell layers Fujii *et al*. (1999b). Various orchid DNA C-values may be accessed online (Bennett and Leitch 2003). Anomalous nuclei, and even plastid DNA nucleoids, can be visualized with *in situ* DAPI staining (Albert 1990).

Phalaenopsis, a monopodial orchid, was the first orchid genus to be propagated *in vitro* while *Cymbidium* was the first to be propagated by shoot-tip, and a comprehensive analysis of conditions required for the tissue culture and micropropagation of various orchids has been reviewed elsewhere (Arditti and Ernst 1993).

Both solid and liquid media are suitable for orchid proliferation, which is generally faster and more extensive in the latter, both shaken and stationary, except for *Paphiopedillum* (Arditti and Ernst 1993). Agitation may be desirable to proliferate certain cultures in order to eliminate polarity, or to retard shoot or root development (Scully 1967; Wimber 1963), improved aeration, increased surface area, accelerated dilution of toxic metabolites (Ishii *et al*. 1976a, 1979a), or stimulation of PLB formation.

Most orchid tissue cultures require PGRs for growth, callus or PLB formation, proliferation and development (**Table 1**), and even though some early attempts at tissue culture of orchids failed due to the lack of PGRs in the media, other studies involved the successful growth of orchids without the use of PGRs (Morel 1960; Wimber 1963). In terrestrial *Cymbidium*, it is well known that auxins stimulate rhizome growth while cytokinins induce upright shoot formation (Hasegawa *et al*. 1985; Paek and Yeung 1991; Lu *et al*. 2001). In *Campylocentrum burchelii*, a shootless orchid, show a high biosynthetic capacity for endogenous indole-3-acetic acid (IAA) and cytokinins in a single organ, with the IAA/cytokinin ratio favourable to auxin in the roots (Peres *et al*. 1997). The antiauxin, *trans*-cinnamic acid (*t*CA) is used to release apical buds from their dormant state, allowing them to develop into shoots (Mosich *et al*. 1974). In addition to various auxins and cytokinins, coconut water is commonly used in the tissue culture of orchids (**Table 1**), although it inhibited *Cattleya lawrenceana* cultures (Mariat 1951). Coconut liquid endosperm contains a large spectrum of biochemicals that can act as growth factors individually or synergistically (Shantz and Steward 1952), such as 1,3-diphenylurea, which shows cytokinin-like activity, and zeatin riboside and zeatin (Letham 1974; Dix and van Staden 1982). In studies conducted on *Dendrobium*, *Oncidium* and *Cattleya*, it was uniformly found that PGRs showed a ranking with respect to their capacity to produce PLBs: 6-benzylaminopurine (BAP) > kinetin > 1-naphthaleneacetic acid (NAA) > IAA > 2,4-dichlorophenoxyacetic acid (2,4-D) > gibberellic acid (GA_3) (Saiprasad *et al*. 2002). BA will be used as used in the original reference throughout the review, even though they represent the same PGR (Teixeira da Silva 2012). Chowdhury *et al*. (2003) showed that BAP together with NAA were necessary for callus induction in *Doritaenopsis*, while NAA enhanced the formation of PLBs from callus. Similar positive callus and PLB stimulating effects of BAP and NAA were obtained for *Dendrobium formosum* (Nasiruddin *et al*. 2003) and *Cymbidium pendulum* (Vij *et al*. 1994).

Without exception, all orchid tissue culture and micropropagation studies used sucrose as the carbon source (2-5%), although a number of other carbohydrates such as glucose or fructose have been shown to be suitable for *Phalaenopsis* and *Dendrobium* (Ernst 1967; Hew *et al*. 1988); others, particularly galactose, were shown to be toxic (Ernst *et al*. 1971). A high sucrose concentration (5%) was shown to almost double the number of PLBs formed on a Hyponex® medium (Momose and Yoneda 1989). Photoperiod, pH of the medium and growth temperature requirements are very similar for many orchids (Vacin and Went 1949; Arditti and Ernst 1993). There are endless choices of macro- and microelements, while the advantage of adding amino acids is questionable. In terms of vitamins, the addition of niacin to the medium slightly improved *Cymbidium* growth (Fonnesbech 1972b) while Koch (1974) found that *Phalaenopsis* PLBs could only be cultured for an extended time if the medium contained pantothenic acid, niacin, pyridoxin, thiamine, and glycine. The polyol *myo*-inositol, a constituent of coconut liquid endosperm, and involved in cell-wall biosynthesis, is often added to tissue culture media, where it was shown to improve *Cymbidium* growth (Fonnesbech 1972a). The sugar alcohol mannitol is widely used to prepare isotonic media for the culture of protoplasts. In the tissue culture of *Dendrobium* and *Darwinara*, sorbitol and mannitol were detected in the medium following one month PLB micropropagation (Kishi and Takagi 1997b). A high sucrose concentration (10%) or a high ABA concentration (10 mg l^{-1}) resulted in tolerance in *Spathoglottis plicata* protocorms, the former resulting in sucrose accumulation, the latter in dehydrin accumulation (Wang *et al*. 2003).

Organogenic media often demonstrate genotype-dependence (**Table 1**), as in PLB-inducing medium in *Phalaenopsis* (Tanaka 1992), *Cattleya* (Ichihashi and Kako 1973), and *Cymbidium* (Kano 1972). Often seeds are surface-sterilzed prior to plating on the appropriate germination medium, but benomyl, a systemic fungicide, was found to be phytotoxic to seedlings (Gupta and Hadley 1977). Knudson C medium was the best medium for seed germination in *Spathoglottis plicata* (Singh 1992), *Cymbidium* (Torikata *et al*. 1965), *Cattleya* (Boesmann 1962), and *Dendrobium* (Mowe 1973), while the presence of NAA in the medium enhanced germination in *Cattleya*, *Cymbidium* and *Bletilla* (Strauss and Reisinger 1976). In contrast, Alam *et al*. (2002) found MS medium to be superior to Hyponex®, Knudson C or OKF_1 in *Dendrobium transparens* seed germination.

Some smaller flowering species in the section *Zebrinae*, such as *Phalaenopsis lueddemanniana* usually produce plantlets (*keikis* or off-shoots) on their flower stalks or roots (Brasch and Kocsis 1980; Arditti and Ernst 1993; Smeltz 1995; Wang 1995). In *Aranda* Deborah, decapitation results in induced flowering (Goh and Seetoh 1973), while the exogenous application of BA stimulated flowering in mature *Dendrobium* pseudobulbs, although decapitation did not (Goh and Yang 1978; Goh 1979).

The inclusion of tryptone in the medium improves callus regeneration and proliferation (Huan *et al*. 2004). Extract from fungus-containing rhizomes enhanced growth and development of some *Cymbidium* explants (Ueda and Torikata 1974), while the bleeding sap of birch trees enhanced the growth of *Brassia*, *Cattleya*, and *Cymbidium* explants (Zimmer and Pieper 1977). Banana powder often enhances seedling growth from immature embryos of *Vanilla* (Withner 1955), *Phalaenopsis* and *Paphiopedilum* (Ernst 1975) while the immersion of entire *Brassolaeliocattleya*, *Dendrobium*, *Epidendrum*, *Laeliocattleya* and *Phalaenopsis* seedlings in 2-(3,4-dichlorophenoxy)triethylamine (DCPTA) enhanced their total fresh weight and growth (Keithly and Yokoyama 1990; Keithly *et al*. 1991). Growth of *Brassolaeliocattleya*, *Dendrobium*, *Epidendrum*, and *Phalaenopsis* seedlings was enhanced (2-3-fold) by the addition of 30 μM DCPTA (Keithly and Yokoyama 1990; Keithly *et al*. 1991). Addition of activated charcoal (AC), usually vegetable in origin (Yam *et al*. 1990), to the media allows for favourable *Cymbidium*, *Phalaenopsis*, and *Paphiopedilum* seed germination *in vitro* (Yam *et al*. 1989). Browning of *Cattleya* explants due to phenolic production may be reduced by culturing shoot tips at pH 5.5, and incubation of tissues at 15-20°C (Ishii *et al*. 1979a). Two such phenolics, eucomic and hydroxyeucomic acid, often produced in the shoot tip cultures of *Cattleya*, inhibits both shoot and root development (Ishii *et al*. 1976a, 1976b, 1979b). Shoot tips of *Phalaenopsis* are occasionally inhibited in *in vitro* culture, and this has been attributed to the phyto-inhibitory phalaenopsine T (Fujieda *et al*. 1988). The inclusion of AC (0.2 to 3 g l^{-1}) enhanced the growth and development of *Cymbidium* seedlings, and *Paphiopedilum* (Ernst 1974, 1975) and *Phalaenopsis* (Tanaka *et al*. 1988) plantlets. AC (or darkness) in *Cymbidium* shoot tip medium establishes polarity and induces positively geotropic terrestrial roots (Werckmeister 1971). The addition of PVP (polyvinylpyrrolidone) at 800 mg l^{-1} enhanced PLB formation (Tanaka 1987). Morphactins, such as the *n*-butyl ester of 9-hydroxyfluorene-(9)-carboxylic acid stimulate carbohydrate synthesis and shoot formation in *Phalaenopsis* (Koch 1974). Fe-EDTA was effective in shoot and/or root growth of *Cymbidium* Water King, *Calanthe discolor*, as well as inhibiting leaf browning symptom in *Bletilla striata* (Paek *et al*. 1993). The presence of certain *Abies*, *Annona*, *Calocedrus*, *Ficus*, *Fraxinus*, *Pinus*, *Pseudotsuga*, *Quercus*, *Sequoia*, or *Taxodium* bark substrates, viz. ellagic and other gallic acid derivatives, leucoanthocyanin tannins, among others were shown to inhibit epiphytic orchid germination (Frei and Dodson 1972; Frei 1973; Frei *et al*. 1975).

An important study on the effects of plant hormones on organogenesis of *Cymbidium* shoot apices *in vitro* (Kim and Kako 1982, 1983) concluded that: a) auxins induce roots on shoot apex explants but inhibit shoot development; b) the fresh weight of plantlets increases in the presence of high levels of 2,4-D, but roots are abnormal; c) NAA at 1 g l^{-1} or 2,4-D at 0.1 g l^{-1} can enhance plantlet formation and development; d) BA can enhance the formation of PLBs and shoots, but at high concentrations it inhibits root initiation; e) GA and ABA have no effect; f) explants with three leaf primordia do not require an exogenous suply of PGRs for development; g) the production of PLBs is seasonal, with production beginning to increase in April, and reaching a peak in June; PLBs form in both the axillary and basal regions. In separate studies on *Cymbidium* (Kukułczanka and Paluch 1971; Kukułczanka and Twarda-Predota 1973; Morawiecka *et al*. 1973; Kukułczanka and Jastrzebska-Kolodynska 1976-1977; Kukułczanka 1985; Kukułczanka *et al*. 1987; Kukułczanka *et al*. 1989) the following was concluded: peptone enhances shoot tip cultures of *Cymbidium* when added to a modified Tsuchiya medium at a concentration of 2 g l^{-1}; b) addition of auxin after 4 weeks of culture is advisable since it enhances the growth of PLBs and plantlets; c) kinetin increases the number of PLBs; d) GA accelerates shoot growth but inhibits root development; e) morphactin at 0.01-10 mg l^{-1} can increase the number of PLBs, but it could also induce developmental anomalies in rhizoids, shoots and roots; f) the addition of microelements and magnesium enhanced the growth of PLBs; g) addition of streptomycin to culture media brought about changes in the activity and electrophoretic patterns of PLB-derived acid phosphatase and ribonuclease; h) the addition of Biostimin, an *Aloe* extract, improved the colour, fresh and dry weight, and number of PLBs. Despite the positive effect of PGRs in *Cymbidium* organogenesis, Laneri (1990) and Hasegawa (1991) claim that their presence causes phenotypic variations in the propagules. The use of 2,4-D (1-3 mg l^{-1}) in *Spathoglottis plicata* produced callus or vigorous shoot growth (Singh 1992), while only callus was observed in *Cymbidium* (Ueda and Torikata 1969; Fonnesbech 1972a). Oyamada (1989) showed that a high NO_3^-:NH_4^+ resulted in as much as a 10-fold increase in fresh weight when *Cymbidium* PLBs were placed in liquid shake culture. High Cl^- and H^+ concentrations positively affected seedling regeneration in *Phalaenopsis* hybrid while increased NH_4^+ and NO_3^- concentrations promoted shoot growth (Hinnen *et al*. 1989). The slow growth of orchids was attributed to the sluggish nitrogen metabolism (Poddubnaya and Arnold 1967), and the supply of calcium nitrate to *Dendrobium wardianum* cultures resulted in direct shoot formation, surpassing the PLB formation stage, as would comonly occur with all the other nitrogen sources (Sharma and Tandon 1992); urea, although reported by Mariat (1948) to be an effective N source for *Cattleya* embryos, is a necrotic agent at higher concentrations (Sharma and Tandon 1992). Concentrations and ammonium:nitrate ratios in the culture media have been known to affect the germination of orchid seeds and subsequent organogenesis considerably (Rappaport 1954; Lugo 1955; Raghavan 1964; Raghavan and Torrey 1964; Uesato 1973, 1974; Ichihashi and Yamashita 1977; Ichihashi 1978, 1979a, 1979b; Shimasaki and Uemoto 1990).

CELL, TISSUE AND ORGAN CULTURE AND MICROPROPAGATION

This topic has been covered more recently through a different prism by Chugh *et al*. (2009).

The choice of explant source also plays a significant role in the outcome of micropropagation, which, in *Phalaenopsis* can be achieved through the use of shoot tips (Intuwong and Sagawa 1974), root tips (Tanaka *et al*. 1976), axillary buds on the flower stalk (Tse *et al*. 1971; Koch 1974; Fu 1979a), segments of broken plantlets derived from the axillary bud (Pieper and Zimmer 1976; Zimmer and Pieper 1978), as well as internodal sections of the flower stalk (Homma and Asahira 1985; Lin 1986). The use of thin cross sections (TCSs) or thin cell layers (TCLs) of plant

Table 1 Principle orchid regeneration studies (≤2004).

Species, principal cultivar(s) + others	Explant source	Organ	Basal medium**	+/- CW	PGR/additive *1	Reference
Acampe rigida, wild	Leaf tip	PLB/plantlet	H, IY	-	0.2 NAA	Yam and Weatherhead 1991a
Acampe praemorsa	Leaf	Shoot bud	MS	-	1 TDZ→2 NAA 0.5 BA	Nayak *et al*. 1997a
Aëridachnis Apple Blossom + 2	PLB	EC	VW^{m}	+	0.1 NAA 0.01 TDZ	Lim-Ho 1981
Aerides japonicum	Protocorm	Plantlet	Hy	-	0.01 BA; 2 g AC 4 g peptone 0.35% BH	Kim *et al*. 1990
Agrostophyllum myrianthum	Protocorm	Seedling	Ni	-	Na-alginate synseed	Chetia *et al*. 1998
Anacamptis pyramidalis	Meristem	Plantlet	MS	+	2.6 K	Morel 1970
Anoectochilus formosanus	Lateral bud (TCL)	Shoot	MS^{m}	-	0.5 NAA 2 K	Chow *et al*. 1982
		Root	MS^{m}	-	0.5 NAA/2,4-D 3 K	
	Seedling	Plantlet	½MS	-	2 BA 0.5 NAA; 0.2% AC, 8% BH	Nalawade *et al*. 2003
Anoectochilus sikkimensis, *A. regalis*	Node	C/shoot	WPM	-	2.2 BAP→2.7 NAA 0.2% AC	Gangaprasad *et al*. 2000
***3** *Arachnis hookeriana* Capama	Apical/axillary bud	Plantlet	VW^{m}	+	2 NAA; TJ BH	Lim-Ho 1981
MerahMaggie Oei	Lateral bud	C/plantlet	MS^{m}→M_{L}	+	0.5 2,4-D	Vajrabhaya and Vajrabhaya 1976
Arachnostylis	Apical/axillary bud	Plantlet	VW^{m}	+	2 NAA; TJ BH	Lim-Ho 1981
Aranda Wendy Scott Greenfield + 12	Apical/axillary bud	PLB	VW/SH	+	0.25-0.5 NAA + BA	Khaw *et al*. 1978
Wendy Scott, Noorah Alsagoff, Christine	Leaf tip/base	PLB/leaf	VW/MS	-/+	2 2,4-D 2 BA	Lay 1978
				+	0.2 2,4-D/0.5 BA; CH	Cheah and Sagawa 1978
Greenfield	Apical/axillary bud	Shoot/PLB	VW/VW^{m}/ K^{m}	+	2 g Peptone	
Deborah, Christine	Apical/axillary bud, leaf	Shoot/PLB/C	VW_{L}/MS/W			Loh *et al*. 1978; Goh 1981
Christine # 27,130 + 28	Apical/axillary bud, leaf	Shoot/PLB/C	VW_{L}/MS^{m}/	+	2 g Peptone	Fu 1978 1979b
Noorah Alsagoff	Non-green callus	Callus	W/K^{m}	+	NH_4^+ vs NO_3^- effects; agitation + aeration	Lee *et al*. 1987
Tay Swee Eng	Shoot tips	Callus	VW_{L}	+	2.5 g $NaNO_3$	Chia *et al*. 1988
Deborah	Inflorescence shoot tip	Plantlet	VW_{L}	+	1 BA 1 K	Goh and Wong 1990
Deborah	Shoot tip TCS	PLB/plantlet	K_{L}→VW_{L} VW	+	0.2-1 NAA 0.2-2 BA; 0.5 g AC	Lakshmanan *et al*. 1995b
Aranthera James Storei	Apical/axillary bud	Shoot/PLB	VW/VW^{m}/K^{m}	+	0.2 2,4-D/0.5 BA; CH	Irawati *et al*. 1977; Cheah and Sagawa 1978 Ong and Chua 1978
Aranthera Beatrice Ng Conference Gold	Axillary bud	Plantlet?	K^{m}/VW^{m}	-	1 NAA 0.2 2,4-D 0.2 BA	Irawati 1978
Arundina bambusifolia Hilo Rose	Meristem, leaf	PLB/plantlet	VW_{L}/MS^{m}/ W/K^{m}	+	2 g Peptone; BH	Mitra 1971; Banik *et al*. 1986
Arundina graminifolia	Seedling	Plantlet	K			Nishimura 1991
Ascocenda 50th State Beauty	Leaf	PLB/plantlet	MPR	-/+	BAP/K/IAA	Vij and Kaur 1999
Ascocenda Hilo Rose x *Vanda* Josephine	Leaf tip/base	PLB/leaf	MS	+	2 2,4-D 2 BA	Lay 1978
Ascocentrum ampulaceum	Floral bud	PLB	†§ MS→Hy→ MS	-	NH_4^+ vs NO_3^- effects	Kishi and Takagi 1997a
Ascofinetia Cherry Blossom	Inflorescence	PLB/plantlet	VW	+	PH; BH	Intuwong and Sagawa 1973
	Floral bud	PLB	†§ MS→Hy→ MS	-	NH_4^+ vs NO_3^- effects	Kishi and Takagi 1997a
Bletia purpurea	Seedling	Plantlet	K			Nishimura 1991
Bletilla striata, wild	Root tip	Plantlet	MS^{m}/Kn	-	1 NAA 0.2 BA; 3000 Gr	Ichihashi and Yamashita 1977
	Immature seed	SE	§ H/VW	-	3 2,4-D 1 K	Lee *et al*. 1990
	Stem node	Shoot/plantlet	Kn^{m}	-	70 tCA	Yam and Weatherhead 1990, 1991a
	Seedling	Plantlet	K			Nishimura 1991
	Root tip	Shoot/plantlet	Kn^{m}	-	70 tCA	Yam and Weatherhead 1991b
						Paek *et al*. 1993
					Uniconazole, paclobutrazol, ancymidol	Chung *et al*. 1999
Brassia rex Sakata FCC/AOS x *B. verucosa*	Protocorm	PLB	Phytamax-$Sigma_{L}$	-	2 g AC	Knapp *et al*. 2000
Brassocattleya Pastoral Innocense BM/JOGA	Axillary bud	PLB	†§ MS→Hy→ MS	-	-	Kishi and Takagi 1997
Brassocattleya Princess Patricia	Axillary bud	C/plantlet	MS^{m}	-	0.1 NAA→10 2,4-D 10 K	Kako 1973
Brassolaeliocattleya	Plantlet	Rooted plantlet	MS^{m}	-	Culture on polypropylene rafts	Adelberg *et al*. 1992
***17**	Seedling leaf	Plantlet	K^{m}	-	3-5 BA; 10% BH	Ding *et al*. 2002
Burkillara Ong Thye Chiew	Apical/axillary bud	Plantlet	MS^{m}/VW^{m}	+	2 NAA; TJ BH	Lim-Ho 1981
Calanthe discolor						Paek *et al*. 1993

Table 1 (cont.)

Species, principal cultivar(s) + others		Explant source	Organ	Basal medium**	+/- CW	PGR/additive *1	Reference
Catasetum (*C. trulla* x *C.* Berthrand)		Root tip	Plantlet	K^m	+	5 NAA; 1 g peptone	Kerbauy 1984a
Catasetum fimbriatum		4-month seedling root tip	PLB/plantlet	K/LS	-	0.5-8 IAA/IBA/2,4-D 0.5-8 BA; 60 g BH; 1 g AC	Colli and Kerbauy 1993
		Seedling root tip	Plantlet	VW^m	-	60 g BH; 1 g AC	Vaz *et al*. 1998
		Stem	Plantlet	‡ VW^m/MS	-	-	Majerowicz *et al*. 2000
Catasetum pileatum		Root apex	PLB	MS	-	1 g peptone 1 g AC	Kraus and Monteiro 1989
Cattleya	n.s.	Lateral bud	Shoot	RM_L→RM	+	1.75 NAA 1.75 IBA→1 K	Reinert and Mohr 1967
	n.s.	Vegetative shoot	C/PLB/plantlet	$K^m{}_L$→VW	+	0.1 K 2 IAA	Scully 1967
	C. bowringiana x *C. forbesii*	Leaf base	PLB/plantlet	H^m	-	1 K	Champagnat *et al*. 1970
	n.s.	Meristem	C/root/plantlet	† $MS^m{}_L$/$K^m{}_L$	+	1 NAA 0.2 K→0.2 NAA 0.35 GA_3 0.2 K; CH	Lindemann *et al*. 1970
	n.s.	Backbulb dormant bulb	C/PLB/plantlet	H^m→MS→ LS→K^m	+/-	1 2,4-D <1 BAP→1 NAA 3 K→0.1 K 2 IAA; BH	Churchill *et al*. 1971
	Princess Margaret, Bonanza Giant	Protocorm-seedling	Plantlet	K/Ni	-	NH_4^+ vs NO_3^- effects	Uesato 1973
	Dorothy Fried No. 1	Apical/axillary bud	Plantlet	MS	-	0.1-1 K 1-5 NAA/0.1 2,4-D; BH	Kusumoto 1978, 1979b
	n.s.	Leaf tip	PLB/plantlet	K^m	+/-	1 2,4-D	Vajrabhaya 1978
	Dorothy Fried No. 1	Shoot	Protocorm	MS	+	5 BA 0.1 NAA; BH	Kusumoto 1979a
	C. labiata	Callus	Protocorm	RM	-	Na_2Fe-EDTA (varying levels)	Stoltz 1979
	n.s.	Shoot	Shoot/root	MS^m	+	0.1 NAA 10 AS 1 BA→0.1 NAA 30 AS 1 BA	Huang 1984
	***13**	Root tip	C/PLB/root	VW	+/-	0.1 24-D 0.05 BA 0.5 NAA	Kerbauy 1991
	Georgiana x self	Plantlet	Plant	MS/K/L/VW	-	0.3 NAA	Adelberg *et al*. 1992, 1997
	C. aurantiaca	Shoot tip	Shoot/PLB	MS/Phyta-max-Sigma	- -	10 BA 0.1 NAA; 100 g BH 2 AC	Mauro *et al*. 1994
	C. leopoldii	Embryo	LB	Phytamax-Sigma	-	2 g AC	Knapp *et al*. 2000
	n.s.	Fractionated PLB	PLB	MS		1 BA	Saiprasad *et al*. 2002; Saiprasad and Polisetty 2003
Cleisostoma fordii		Leaf tip	PLB/plantlet	H, IY	-	0.2 NAA	Yam and Weatherhead 1991a
		Root tip	Shoot/plantlet	Kn^m	-	70 tCA	Yam 1989; Yam and Weatherhead 1991b
Coelogyne odoratissima var. *angustifolia*						Encapsulated protocorms	Kamalakannan *et al*. 1999
Cymbidium	sp.	Shoot meristem	PLB/plantlet	† /K^m/VW^m	-	-	Morel 1960, 1970; Sagawa *et al*. 1966; Wimber 1963
	sp.	Leaf tip callus	Plantlet	†	-	BH/2000 tryptone	Arditti *et al*.1971; Churchill *et al*. 1973
	sp.	Shoot tip/callus	C/PLB/plantlet	W	+	5 NAA 3 g G	Steward and Mapes 1971
	sp.	Leaf tip/protocorm	Plantlet	† Wi	-	1.9 NAA 0.2 K; 3-4 g tryptone	Fonnesbech 1972a, 1972b
	sp.	Dormant bud	PLB/plantlet	MS^m	+	1.9 IAA 2.2 K→1.9 IAA 2.3 BA	Tran Thanh Van 1974a, 1974b
	16 hybrids	Shoot tip	PLB/plantlet	K^m	-	-	Dalla Rosa and Laneri 1977
	sp.	Shoot apex	PLB/plantlet	K^m	-	BH AJ 2 g peptone	Kusumoto and Furukawa 1977; Kusumoto 1980
	sp.	Apical/axillary bud	PLB/plantlet	VW^m	-	BH	van Rensburg and Vcelar 1984
	sp.	Shoot tip	PLB/plantlet	K^m	+	1 BA	Gu *et al*. 1987
	sp.	Apical meristem	PLB/plantlet	K_L	-	2 NAA 1 BA	Yang *et al*. 1999
	sp.	Immature seed	Seedling	VW	+	0.1 NAA; 2.5 folic acid CH peptone	Bannerjee and Mandal 1999
Cymbidium aloifolium		Shoot	Shoot/root	MS	-	1 BA 1-2 TDZ→1 BA 2 NAA→2	Nayak *et al*. 1997b
		Rhizome (from seed)	Shoot/root	MS	-	IBA	Nayak *et al*. 1998a
		PLB thin cross section	PLB/shoot	MS	-	5 NAA→1 BA 0.1 NAA→1 NAA 2 ZR→2 IBA	Nayak *et al*. 2002
Cymbidium gyokuchin var. *soshin* Kwanum							Paek *et al*. 1993
Cymbidium ensifolium		Axillary/flower bud	Protocorm/root	MS	-	4 BA 0.1 NAA 1 GA_3	Jia *et al*. 2000
Cymbidium ensifolium, *C. goeringii* Qiulan		Apical/axillary bud	Plantlet/flower	MS^m/W^m	+	2.5/5 NAA [2 BA] 3 G	Wang *et al*. 1981, 1988; Wang 1988b
Cymbidium ensifolium var. *misericors*		Pseudobulb/rhizome/root	C/SE/rhizome	‡ MS	+	3.3 2,4-D 0.33 TDZ; 1 g peptone	Chang and Chang 1998
Cymbidium ensifolium Yuh Hwa		Rhizome apex	Rhizome/shoot	MS	-	6 NAA→2 NAA; 2 g AC (horizontal shaker)	Lu *et al*. 2001
Cymbidium faberi		Rhizome tip	Shoot	§ $Kyoto^m$	-	0.5 NAA 1 BA 3 g tryptone 2 g AC	Hasegawa *et al*. 1985
***3** *Cymbidium faberi*, *C. forrestii*, *C. goeringii*, *C. hakuran*, *C. insigne*, *C. kanran*, *C. sinense*		Shoot tip	Rhizome	§ MS/LS/Hy	-	1 NAA 1 K	Hasegawa *et al*. 1987a, 1987b

Table 1 (cont.)

Species, principal cultivar(s) + others	Explant source	Organ	Basal medium**	+/- CW	PGR/additive *1	Reference
Cymbidium forrestii Garyung	Rhizome (from seed)	Shoot	MS	+	1 NAA 5 BA/10 BAP; AC 3% BH	Paek *et al.* 1990, 1991; Paek and Yeung 1991
Cymbidium giganteum	Shoot tip	Plantlet	MS	-	0.5-5 NAA; 0.5-5 BAP (Na-alginate synseed)	Corrie and Tandon 1993
Cymbidium goeringii Reichenbach	Apical flower bud	Rhizome/plant	MS^{m}	-	0.1 BA 10 NAA	Shimasaki and Uemoto 1991
Cymbidium goeringii Qiulan, *C. insigne*	Shoot	Root	K^{m}	-	10 K; effects of light: white vs. blue vs. red	Ueda and Torikata 1968, 1969a, 1969b, 1972, 1974
Cymbidium goeringii, *C. kanran*	Shoot tip	Rhizome	MS/LS	+	1 NAA 0.1 K	Hasegawa and Goi 1987b; Shimasaki and Uemoto 1991
Cymbidium insigne Rolfe, *C. eburneum* Lindley	Shoot	PLB/shoot/root	‡ MS^{m}	-	1% quartz porphyry “bakuhan-seki”	Shimasaki *et al.* 2003a, 2003b
Cymbidium kanran Makino	Rhizome	Shoot/root	K/MS	-	0.2-2 NAA 0.1-0.2 K	Kokubu *et al.* 1980
	Rhizome	Shoot	MS^{m} ***A**	-	-	Lee 1986
	Rhizome	Shoot/root	MS/Hy	-	0.1 NAA 5-10 BA; 4 g peptone 0.1% AC	Kim *et al.* 1988
	Rhizome	Shoot/root	MS^{m}	-	NAA BAP; No PGR = low NH_4^+ and KNO_3	Shimasaki and Uemoto 1990
Cymbidium lancifolium	Rhizome segment	Shoot/root	MS^{m}	-	1 g AC	Kim and Lee 1992
Cymbidium longifolium	Protocorm	Seedling	Ni	-	Na-alginate synseed	Chetia *et al.* 1998
Cymbidium niveo-marginatum						Lee 1988a, 1988b
	Pseudobulb/seed→rhizome	Plantlet	MS^{m}	-	-	Shimasaki and Uemoto 1990
Cymbidium pendulum					BAP NAA	Vij *et al.* 1994
Cymbidium sinense Willd	Rhizome (from seed)	Shoot	‡ MS	-	0.5 NAA; 1 g peptone 1 g AC	Chang and Chang 2000
Cymbidium virescences						Lee and So 1985
Cymbidium x Emken (hybrid)			MS			Prasad and Verma 2001
Cymbidium April Rose, Tsurugi Awabijin, Waltz SS	Shoot tip	PLB	MS/Hy	-	20/35/50 g/l sucrose	Momose and Yoneda 1989
Cymbidium Lancelot Yagoto + Misono	PLB	Plantlet	Hy	-	0.1 2,4-D/NAA + 1/5/10 K/GA	Kusumoto 1981a
Cymbidium Lois Kelly Cherry, Gawain Empress	PLB	Shoot	VW	-	0.1 NAA 0.1 K; 2 g tryptone in Culture Pack®	Tanaka 1991
Cymbidium Melody Fair ‘Marilyn Monroe’	PLB	Shoot	MS	-	Effects of sugar uptake and shoot formation	Ogasawara *et al.* 1995
Cymbidium Reporsa	2-3 leaves + roots	Plantlet	Hy	-	CO_2-enrichment	Kozai *et al.* 1990
Cymbidium Thanksgiving cv.	PLB outer tissue	PLB	MS_L	-	2 NAA 1 BA	Fujii *et al.* 1999a 1999b
Nativity, *C.* Lucky Rainbow Lapine Dancer	PLB inner tissue	EC	MS_L	-	1-2 NAA 0.1-2 2,4-D	Begum *et al.* 1994a 1994b
Cymbidium Twilight Moon ‘Day Light	PLB	EC	VW^{m}	+	0.1 NAA 0.01 TDZ	Huan *et al.* 2004
Cymbidium Water King						Paek *et al.* 1993
Cypripedium macranthos var. *rebunense*	Seed (mature)	PLB	Hy	-	0.2 NAA; 2 g peptone	Shimura and Koda 2004
Cypripedium macranthos var. *taiwanianum*	Seed-derived callus	Plantlet	TT	-	1 NAA 0.1 BAP	Tomita and Tomita 1997
Cypripedium montanum	Protocorm node	Rhizome/shoot	MS	-	0.2 NAA 2 BAP	Hoshi *et al.* 1994
Cyrtopodium cf. *punctatum*	Root tip	PLB/plantlet	VW^{m}	+	0.5-1 NAA 0.1-0.5 BA; 1 g peptone	Sánchez 1988
Dactylorchis fuchsii	Shoot tip/floral primordia	Protocorm/plant	K/RM^{m}	+	0.1 NAA 0.2 K	Roy and Banerjee 2003
Dactylorhiza incarnata	Protocorm	Protocorm	Original	-	Starch agar; 50 ml birch sap + *Rhizoctonia* spp.	Beyrle *et al.* 1991
Darwinara Pretty Girl	Floral bud	PLB	†§ MS→Hy→ MS	-	NH_4^+ vs NO_3^- effects	Kishi and Takagi 1997a 1997b
Dendrobium sp.	Seedling	Plantlet	VW^{m}	-	Kappa carrageenan (from *Euchema cottonii*)	McConnell and Tomomitsu 1983
Dendrobium candidum	Protocorm	Protocorm/ shoot			BA + NAA; spermine	Wang *et al.* 1997
Dendrobium densiflorum	Node	PLB	MPR	-	1 BA 1 NAA (Na-alginate synseed)	Vij *et al.* 2001
Dendrobium moniliforme	Protocorm	Plantlet	Hy	-	1: GA/NAA/IBA/K/ABA	Lim *et al.* 1993
	Floral bud	PLB	†§ MS→Hy→ MS	-	NH_4^+ vs NO_3^- effects	Kishi and Takagi 1997a 1997b
Dendrobium nobile	Protocorm-seedling	Plantlet	K/Ni	-	NH_4^+ vs NO_3^- effects	Uesato 1974
Dendrobium phalaenopsis	Flower stalk	Plantlet	VW^{m}	-	-	Intuwong *et al.* 1972
	Stem node	PLB/plantlet	Kn^{m}	-	2 BAP 1.5-150 tCA → 0.1 IAA	Mosich *et al.* 1974
	Seedling tip	Bud/root	MS^{m}	-	-	Gandawijaja 1980
	***3** Apical/axillary bud	Shoot/root	VW^{m}/K^{m}	-	0.2 NAA/ 0.3 IAA	Lim-Ho 1981***2**
	Shoot tip	Plantlet	† /K^{m}/VW^{m}	+	-/+	Sagawa and Kunisaki 1982; Kim *et al.* 1970

Table 1 (cont.)

Species, principal cultivar(s) + others	**Explant source**	**Organ**	**Basal medium****	**+/- CW**	**PGR/additive *1**	**Reference**
Dendrobium phalaenopsis, *D. antennatum*	Axillary bud	C/PLB/plantlet	RM^{m}	-	1.75 IBA 1.75 NAA 5 BA; 1 g peptone	Kukułczanka and Wojciechowska 1983
Dendrobium phalaenopsis Banyan Pink	Germinated seed	C/PLB	MS	-	1BA	Men *et al.* 2003
D. aduncum, *D. loddigesii*, *D. transparens*	Stem node	Shoot/plantlet	Kn^{m}	-	70 tCA	Yam 1989; Yam and Weatherhead 1990
Dendrobium aphyllum, *D. moschatum*	Shoot	Shoot/root	MS	-	1 BA 1-2 TDZ→1 BA 2 NAA→2 IBA	Nayak *et al.* 1997b
Dendrobium candidum	Seed/protocorm	C/floral bud	MS	-	0.3 NAA→2 BA 0.5 NAA; 0.5 ABA→2 BA	Wang *et al.* 1990, 1995
Dendrobium chrysanthum	Pseudobulb segment	C/shoot/plantlet	MS^{m}	-	1 NAA 1 K	Vij and Pathak 1989
Dendrobium crumenatum	Seedling leaf	PLB/plantlet	VW^{m}	-/+	5 2,4-D 5 NAA [5 BA]; 150 CH	Manorama *et al.* 1986
Dendrobium fimbriatum var. *oculatum*	Shoot tip	C/plantlet	K^{m}	+	0.5 NAA/1 2,4-D 1 BAP; BH	Stokes 1974; Roy and Banerjee 2003
Dendrobium formosum	Leaf	C/PLB	MS	-	1 2,4-D→2.5 BAP 1 NAA	Nasiruddin *et al.* 2003
Dendrobium linawianum	Lateral bud	Adventitious	MS	-	3 BA 0.2 NAA 3% sucrose	Nalawade *et al.* 2003
Dendrobium macrostachyum	Node	Axillary shoot	MS	+	2.69 NAA 2.22/4.44/8.88 BA/2.32(x2/x3) K	Pyati *et al.* 2002
Dendrobium moniliforme						Lim *et al.* 1993
	Floral bud	PLB	†§ MS→Hy→ MS	-	-	Kishi and Takagi 1997a 1997b
Dendrobium moschatum	Shoot stem disc	PLB	VW^{m}L/ K^{m}L	+	1 2,4-D/2IAA→3 BAP 2 NAA	Kanjilal *et al.* 1999
Dendrobium nobile	PLB thin cross section	PLB/shoot	MS	-	2 BA→2 IBA	Nayak *et al.* 2002
Dendrobium wardianum	Shoot apex	PLB	MS	-	2.5 BAP (Na-alginate synseed)	Sharma and Tandon 1991; Sharma *et al.* 1992
Dendrobium Alice Spalding (*D.* tokai x *D. undulatum*)	Leaf base	Plantlet	VW^{m}	+	-	Fu 1978 1979b
	Axillary bud	Plantlet?	K^{m}/VW^{m}	-	1 NAA 0.2 2,4-D 0.2 BA	Irawati 1978
	Leaf tip/base	PLB/leaf	MS	-	1 2,4-D 0.5 BA	Lay 1978
Dendrobium Caesar Red	Apical/axillary bud	PLB/shoot/ plant	VW^{m}	-	4 NAA	Fernando 1979
Dendrobium Golden Wave	Keiki (off-shoot)	PLB	VW→VW^{m}	+	-	Intuwong and Sagawa 1975
Dendrobium Jaquelyn Thomas White	Shoot tip	PLB/plant	VW^{m}	+	10 NAA	Soediono 1983
Dendrobium Madame Pompadour	Apical meristem	C/PLB/plantlet	MS/VW	+/-	0.2 2,4-D 0.2 BAP; AC	Mujib and Jana 1994
Dendrobium Madame Thong-In	PLB thin section	C/PLB/plantlet	K^{m}L	+	2 BA	Yu and Goh 2000; Yu *et al.* 2001
Dendrobium MiHua	Protocorm	PLB/shoot/ plant	MS	-	5 Z	Yu *et al.* 1999
Dendrobium Miss Hawaii	Scape node	Plantlet	MS^{m}	+	2 BAP; 0.2 g AC	Nuraini and Shaib 1992
Dendrobium Multico White	Apical meristem	Callus	VW	-/+	Different levels of glucose, fructose, sucrose	Sivasubramaniam *et al.* 1987; Hew *et al.* 1988; Hew and Mah 1989
Dendrobium Ng Eng Cheow	Shoot tip	PLB/shoot/ plant	VW^{m}	+	0.2 NAA→4 NAA	Singh 1976
Dendrobium Sonia (*D.* Caesar x *D.* Tomie Drake)	Shoot tip/fractionated PLB	PLB	‡ MS	-	1BAP 2 NAA	Saiprasad *et al.* 2001/2/3; Saiprasad and Polisetty 2003
	Apical meristem	PLB/plantlet	MS	-	0.1 BAP 1 NAA	Prasad *et al.* 2001
	Inflorescence tip/PLB	PLB/plantlet	K^{m}L	+	4 BA	Yang *et al.* 2002, 2003
Dendrobium Sonia 17	PLB	C	‡ MS	-	-	Tee *et al.* 2003
Dendrobium White Angel						Chia *et al.* 1995
Dendrobium White Fairy 1	Plantlet	Plantlet	VW	+	CO_2 enrichment	Lim *et al.* 1992
Disa uniflora	Shoot tip	Plantlet	TGD	-	-	Haas 1977a
	Shoot tip	Plantlet	n.s.	-	0.1 IBA 0.1 BA→0.2 IBA	Van Waes and de Geest 1983
Diuris longifolia	Inflorescence	PLB/plantlet	Burgeffs^{m} N3f	-	0.05 % AC	Collins and Dixon 1992
Doriella (*Doritis* x *Kingiella*)	Vegetative bud	Floral bud	Hy→VW→ Hy	+	5 BA/Z	Duan and Yazawa 1994b
Doritaenopsis sp. (pink)	Flower stalk internode	C/PLB	VW^{m}	-	1/5 BA 1 NAA 2 g AC	Lin 1986
Doritaenopsis (*Doritis* x *Phalaenopsis*)	Flower stalk bud	PLB/plantlet	NDM		1 BA 0.1 NAA	Tokuhara and Mii 1993; Tsukazaki *et al.* 2000
	Flower stem section	PLB/shoot	XER	-	1 TDZ	Ernst 1994
	Flower stalk	C/PLB	NP	-	5 BAP 0.1 NAA→0.5 BAP	Chowdhury *et al.* 2003
Doritaenopsis Coral Fantasy x *Phalaenopsis*	Cell clump	C	NDM	-	1 BA 0.1 NAA	Belarmino and Mii 2000
Doritaenopsis Elizabeth Waldheim	Flower stalk	Plantlet	T	+	150 g AC	Lim-Ho 1981
Doritaenopsis Odoriko	Leaf	PLB	VW^{m}	-	5% PH	Zhou 1995a
Dor. New Candy x *D.* (Mary Anes x Ever Spring	Leaf TCL	PLB	½MS	+	9 TDZ	Park *et al.* 2002

Table 1 (cont.)

Species, principal cultivar(s) + others	Explant source	Organ	Basal medium**	+/- CW	PGR/additive *1	Reference
Doritaenopsis ***9**	Callus	Callus	NP	+	TE, BH, AJ, PH	Ichihashi and Islam 1999
Doritaenopsis ***12**	Embryo/capsule	PLB	Phytamax-Sigma	-	2 g AC	Knapp *et al.* 2000
Doritis pulcherrima	Ovary/immature embryo	Plantlet	VW	+	0.1-10 BA 0.1-10 NAA	Yasugi 1984
Epidendrum radicans O'brienianum	Leaf tip/callus/PLB	C/protocorm	C/MS^{m}	-	1 2,4-D 0.5 BAP	Churchill *et al.* 1970 1973; Arditti *et al.* 1971
	Protocorm/plantlet	Plantlet	HA	-	1 2,4-D 0.5 BAP	Churchill *et al.* 1970
	Root tip	Root	OF^{m}	-	300 g neopeptone	Churchill *et al.* 1972
	Seedling shoot tip	Callus	MS^{m}	-	1-8 OCP	Rudolph *et al.* 1972
	Stem/flower stalk node	Plantlet	MS	+	1 IAA 0.04 K	Stewart and Button 1976
	Root tip	PLB/plantlet	SH^{m}/K/H/L/ OF^{m}	- -	0.5/1 2,4-D	Stewart and Button 1978
	Shoot tip	PLB/plantlet	Kyoto/K	+	0.1 K 1 NAA	Kusumoto 1981b
	Flower stalk cutting	PLB/plantlet	‡ VW	+	1 BA 1 NAA	Singh and Prakash 1982
	Nodal cutting	C/plantlet	K/VW/MS	-	1-2 NAA	Singh 1992
	Flower stalk internode	PLB/plant	MS^{m}		0.45 TDZ→4.44 BA	Chen LR *et al.* 2002
Epiphronitis Veitchii	Shoot tip/stem-flower bud	PLB	MS	+	1 IAA 0.04 K; 1 2,4-D 0.5 BAP	Kusumoto 1981b
Eulophia hormusjii	Seedling rhizome	PLB/shoot/ plant	MPR/ MPR^{m}	-	1 NAA 1 K; 2 g peptone, 2 g yeast extract	Vij *et al.* 1989
Geodorum densiflorum	Undehisced capsule	PLB	K	+	2 BAP 1 NAA; 0.2 g peptone	Datta *et al.* 1999
	Rhizome (*in vitro*) section	Shoot/plantlet	MS/K	-	0.5 NAA 2 BA; 0.1% AC	Sheelavantmath *et al.* 2000
	Protocorm	Rhizome/shoot	MS	+	4 BAP 1 NAA; 2 g peptone	Roy and Banerjee 2002
	Rhizome tips	Multiple shoot	MS	-	2 BAP	Bhadra and Hossain 2003
Haemaria discolor	Stem node	Shoot	K→K$_{L}$	+	-	Teo 1978
Hetaeria	Stem node	Shoot/plantlet	Kn^{m}	-	70 tCA	Yam 1989
Holttumara (*Arachnis* x *Renanthera* x *Vanda*)	Apical/axillary bud	Plantlet	VW^{m}	+	2 NAA; TJ BH	Lim-Ho 1981
Ipsea malabarica	Immature embryo	PLB/seedling	MPR$_{L}$	-	0.5 BAP 1 NAA/IAA; 0.05 CH	Gangaprasad *et al.* 1999
	Bulb (*in vitro*)	Rhizome/plant	MS	-	2 K	Martin 2003; Martin and Pradeep 2003
Kagawara (*Ascocentrum* x *Renanthera* x *Vanda*)	Apical/axillary bud	Plantlet	VW^{m}	+	2 NAA; TJ BH	Lim-Ho 1981
Laelia	Axillary bud	C/plantlet	MS^{m}	-	0.1 NAA→10 2,4-D 10 K	Kako 1973
Laelia cinnabarina	Seedling	Plantlet	MS/H&A	-	-	Stancato and Faria 1996
Laeliocattleya	Leaf tip/callus/PLB	Plantlet	†K	-	1 2,4-D 0.5 BAP	Churchill *et al.* 1973
	Apical/axillary bud	Shoot/root	MS^{m}	+/-	0.1 NAA 10 AS 1 BA→0.1 NAA 30 AS 1 BA	Huang 1984
Laeliocattleya El Cerrito x Spring Fires	Plantlet	Rooted plantlet	MS^{m}	-	Culture on polypropylene rafts	Adelberg *et al.* 1992, 1993
Laeliocattleya John Cunningham	Leaf	C/PLB	H/MS	-	1 2,4-D 0.5 BA	Matos and de Garcia 1991
Liparis nervosa	Stem node	Shoot/plantlet	Kn^{m}	-	70 tCA	Yam 1989; Yam and Weatherhead 1990
Liparis viridiflora	Leaf tip	PLB/plantlet	H, IY	-	0.2 NAA	Yam 1989; Yam and Weatherhead 1991a
Listera ovata	Immature embryo					Rasmussen *et al.* 1991
Ludisia (*Haemaria*) *discolor*	Single node	Shoot	K	-	150 g BH	Teo 1978
Luisia trichorhiza	Leaf	PLB	MS	-	1 IAA 1 BA/BAP; 2 g AC	Vij and Pathak 1988a
Lycaste	Shoot meristem	PLB/plantlet	† /K^{m}/VW^{m}	-	-	Morel 1960
Malaxis acuminata	Leaf tip	PLB/plantlet	H, IY	-	0.2 NAA	Yam 1989
	Stem node	Shoot/plantlet	Kn^{m}	-	70 tCA	Yam and Weatherhead 1990
Miltonia Storm	Shoot tip/stem-flower bud	PLB	MS	+	1 IAA 0.04 K; 1 2,4-D 0.5 BAP	Kusumoto 1981b
Mokara (*Arachnis* x *Ascocentrum* x *Vanda*)	Flower/axillary bud	PLB	MS^{m}/K^{m}/V W^{m}	+/-	2 IAA 2 K 2 AS/BA; BH	Lim-Ho 1981; Lim-Ho *et al.* 1984
Chark Kuan	Young shoot leaf/shoot tip	PLB	VW/VW$_{L}$	+	0.5-2 K	Ghani and Haris 1992; Ghani *et al.* 1992b
	Seed/young leaf	PLB/plantlet	VW	+	1 K	Arditti and Ernst 1993
*Mormodes histrio****10**	Pseudobulb/root	PLB/shoot	K	-	0.2 NAA 0.5-2 BAP	Holters and Zimmer 1990a 1990b
Neofinetia falcata	Callus	Callus	NP	+	TE, BH, AJ, PH	Ichihashi and Islam 1999; Niimi *et al.* 1995
Neostylis Lou Sneary	Inflorescence	PLB/plantlet	VW	+	PH; BH	Intuwong and Sagawa 1973
Neottia nidus-avis	Inflorescence	PLB/plantlet	H^{m}	-	1 K	Champagnat *et al.* 1971
Nigritella nigra, N. miniata	Shoot apex/tuber	Tuber	MS	-	IAA; K	Haas 1977b
Odontioda	Shoot tip	C/PLB/plantlet	† /K^{m}/VW^{m}	-	-	Morel 1960, 1970
Odontoglossum	Apical/axillary bud	C/PLB/plantlet	† /K^{m}/VW^{m}	-	-	Khaw *et al.* 1978
Odontonia	Shoot tip	C/PLB/plantlet	† /K^{m}/VW^{m}	-	-	Morel 1960, 1970
Oncidium	Shoot tip	C/PLB/plantlet	† /K^{m}/VW^{m}	-	-	Morel 1960, 1970
	Apical/axillary bud	PLB	VW^{m}	+	-	Khaw *et al.* 1978
	Apical bud	Plantlet	VW^{m}	+	2 NAA; TJ BH	Lim-Ho 1981
	Root tip					Kerbauy 1984b
Oncidium papilio	Flower stalk tip	PLB/plantlet	K^{m}/ MS^{m}	-	-; 0.5 NAA 0.05 K; 1 g peptone TJ/BH	Fast 1973

Table 1 (cont.)

Species, principal cultivar(s) + others	Explant source	Organ	Basal medium**	+/- CW	PGR/additive *1	Reference
Oncidium varicosum	Root tip	PLB	K^{m}	+	27.8 Fe-EDTA; 1 g AC; 60 g BH	Kerbauy GB 1984 1993a 1993b
Oncidium Goldiana	Axillary bud	Plantlet?	K^{m}/VW^{m}	-	1 NAA 0.2 2,4-D 0.2 BA	Irawati 1978; Li *et al*. 2001, 2002a, 2002b, 2003a, 2003b
Oncidium Mericlones x 6	Lateral bud	PLB/plantlet	G10	-	1 g tryptone 1 g AC 65 g PH	Chen YH *et al*. 2001
Oncidium Sherry Baby OM8	PLB	PLB	‡ G10	-	1 g tryptone 1 g AC 65 g PH	Liau *et al*. 2003; You *et al*. 2003
Oncidium Gower Ramsey	Scape node	Plantlet	VW	+	2 BAP; 0.2 g AC	Nuraini and Shaib 1992
	Shoot tip	PLB	VW→MS	+	200 CH	Bagde and Sharon 1997
	Root tip/stem/leaf	SE/C/PLB	‡ MS	-	0.1-3 TDZ 3-10 2,4-D 1 g peptone	Chen *et al*. 1999; Chen and Chang 2000a, 2002, 2003
	Shoot tip	PLB	MS	-	1 BAP	Saiprasad *et al*. 2002; Saiprasad and Polisetty 2003
	Leaf	SE/shoot	MS	-	1 TIBA	Chen and Chang 2004
Oncidium various***4**	Flower stalk bud	C/PLB/plantlet	MS^{m}	-	0.5 NAA 0.5 2,4-D 2 BA	Lim-Ho and Lee 1987
Ophrys apifera	Seedling	C/PLB/plantlet	P$_{L}$→S→D	-	0.5 IAA 0.5 K; 0.33 TA 1 g CA BH 1 g glycogen	Hoppe and Hoppe 1988
Ophrys fuciflora, *O. apifera*	Tuber section	PLB	MS^{m}	-	0.5 NAA 0.5 K	Morel 1974
Ophrys lutea, *O. fusca*, *O. speculum*	Mature/immature seed	Protocorm	Cu→OCM4/ OCM4^{m}	-	0.5 2,4-D→1.25-5 IAA 0.25-1 BA	Barroso *et al*. 1990
Orchis papilionacea	Ripe/immature seed	Minituber	MS/2xCu/Cu L	+	0.1 Z 0.05 GA3 3 CH	Pedroso and Pais 1994
Otochilus alba	PB	Runner/PB	MS/Phyta-max-Sigma	+	0.5 NAA 2 2-iP	Mukhopadhyay and Roy 1994
Pachystoma senile	Tuber slice	C/PLB	MPR^{m}	-	1 2,4-D→1 IAA; 2 g AC 1 g YE	Vij *et al*. 1983
*Paphiopedilum****5**	Apical/axillary bud	C/root	MS^{m}	+	4 adenine	Bubeck 1973
*Paphiopedilum****15**	Axillary bud	Plantlet	MS/H	-	1/10 BA	Stewart and Button 1977
Paphiopedilum insigne, *P. villosum*, *P. fairieanum*	Apical bud/shoot tip	C/PLB	H^{m}/TGD^{m}	-	1 2,4-D 0.5 BA; 1 Wuchsstoff "66F"	Stewart and Button 1975 1976
Paphiopedilum	Shoot tip	Shoot/plantlet	MS	+	1-10 2,4-D 0.1-1 TDZ	Arditti and Ernst 1993; Huang *et al*. 2001
Paphiopedilum callosum	Shoot/leaf tip	Plantlet	‡ MS^{m}	+	0.1 NAA 3 2iP 30 adenine 100 BA	Huang 1988
Paphiopedilum callosum x *P. lawrenceanum*	Protocorm	C	MS	+	1-10 2,4-D 0.1-1 TDZ	Lin *et al*. 2000
Paphiopedilum philippinense	Stem node	Plantlet	MS^{m}	-	0.1-3 TDZ 3-10 2,4-D	Chen TY *et al*. 2002
(hybrid PH59, PH60)	Leaf	Shoot	§ MS	-	0.1-3 TDZ 3-10 2,4-D	Chen TY *et al*. 2004
Phaius sp.	Shoot tip	C/PLB/plantlet	† /K^{m}/VW^{m}	-	-	Morel 1960 1970; Arditti and Ernst 1993
Phaius tankervilliae	Protocorm (70 d-old)	Shoot	Ni	-	Na-alginate synseed	Malemnganba *et al*. 1996
Phalaenopsis sp.	Flower stalk node	C/shoot	MS/K	-	2 NAA	Tse *et al*. 1971
	Flower stalk	Shoot/plantlet	VW^{m}	-	-	Intuwong *et al*. 1972
	Flower stalk	Shoot/root	K^{m}/REM	-	25 BAP; BH	Ernst 1975
	Node section	Shoot/PLB	Kn^{m}	-	20 BAP 1.48-14.8 tCA	Ball *et al*. 1974-1975
	Flower stalk node	Plantlet	MS/K/Kn	-	15 tCA	Arditti *et al*. 1977
	Flower stalk internode	C/PLB	VW^{m}	-	1/5 BA 1 NAA; 2 g AC	Lin 1985, 1986, 1987
White	Root tip/flower stalk	PLB/plantlet			0.05-0.1 2,4-D; 5% BH	Momose and Yoneda 1988; Yoneda and Momose 1989
***18**	Flower stalk lateral bud	C/PLB	‡ MS	+/-	-	Ichihashi 1992
	Flower stalk bud tip	PLB/plantlet	NDM		1 BAP 0.1 NAA	Tokuhara and Mii 1993, 2001
Pink Leopard 'Petra'	Flower stalk vegetative bud	Adv. shoot	VW	+	5-40 TDZ/10 BA	Chen and Piluek 1995
	Flower stalk node	Adv. shoot	Hy→VW	-	4 BA; 2 g peptone	Duan and Yazawa 1995b; Cardenas and Wang 1998
	Seedling stem-BA-induced					Duan *et al*. 1996
	Flower stalk node→leaf	PLB	MS→Hy$_{L}$/ VW$_{L}$/L$_{L}$	-	3 BA→15 BA 1 NAA; 1% PH (bioreactor)	Young *et al*. 2000
*Phalaenopsis****6**	Flower stalk	PLB/plantlet	VW^{m}	+	-	Intuwong and Sagawa 1974
*Phalaenopsis****7**	Flower stalk node/leaf	PLB/plantlet	MS^{m}	+	2 g G 1 g CH	Haas 1983
Phalaenopsis amabilis, *P.* Callie	Root tip	C/PLB/plantlet	MS^{m}	-	2 G	Tanaka *et al*. 1976
Flynn, P. (White Falcon x Persistent) x Jimmy Hall	Leaf	PLB	MS^{m}/K^{m}	-	1 NAA 10 BAP; 10 adenine 2 g peptone	Tanaka and Sakanishi 1985
	Root tip	PLB	TGD	-	0.05 2,4-D 1/5 K	Yoneda and Momose 1988a
	Pedicel node	PLB	VW	+	-	Yoneda and Momose 1988b
Phalaenopsis Atien Kaala, Blume						Konow and Wang 2001
Phalaenopsis Betty Hausermann	Flower stalk	PLB/shoot	MS^{m}/MS^{m}$_{L}$	- -	1 IAA 1 K 100 tCA/0.5 NAA 2 BAP; 2 g YE 2 g G	Griesbach 1983
	Nodal inflorescence buds	Shoot/root	PGR/lanolin paste		50 tCA 5 BAP	Griesbach 1984
Phalaenopsis Golden Sands 'Canary'	Protocorm	Plantlet	MS	-	2 g peptone; 0.5 colchicine (10 d dark at 26 ºC)	Griesbach 1981, 1985
Phalaenopsis Happy Buddha	Flower stem section	PLB/shoot	XER	-	1 TDZ	Ernst 1994

Table 1 (cont.)

Species, principal cultivar(s) + others	Explant source	Organ	Basal medium**	+/- CW	PGR/additive *1	Reference
Phalaenopsis Jimmy Hall (+ self), *P.* Capitola	Scape node	Plantlet	MS^{m}	+	2 BAP; 0.2 g AC	Nuraini and Shaib 1992
Phalaenopsis Lavender Lady	Leaf	PLB	§VW^{m}/ MS^{m}	-	1 NAA 10 BAP	Tanaka and Sakanishi 1980; Tanaka 1987
P. White Falcon X *P.* Persistent; *P. amabilis*	Flower stalk	Shoot	VW^{m}	+	-	Tanaka *et al.* 1988; Tanaka and Sakanishi 1978
P. stuartiana, *P. amboinensis*	Leaf	PLB	Kyoto^{m}/Hy	+	1 NAA 10 BA; 800 PVP	Tanaka 1992
	PLB	Plantlet	VW^{m}/Hy	-	2 g AC	Tanaka 1992
Phalaenopsis Michelle x Michelle	Protocorm	PLB	MS	+	2iP; BH; 1.5%fructose + sucrose	Lam *et al.* 1991
Phalaenopsis Orchid World	Leaf	PLB	§VW^{m}/ MS^{m}	-	1 NAA 10 BAP	Zhou 1995b (method of Tanaka 1987)
Phalaenopsis P1-6	Shoot	PLB/shoot/root	‡ MS^{m}	-	1% quartz porphyry "bakuhan-seki"	Shimasaki *et al.* 2003b
Phalaenopsis Phyllis Keys x Band Leader	Lateral bud	PLB/plantlet	MS	-	0.5-5 BA 1 NAA	Wang 1989
Phalaenopsis Richard Schaffer Santa Cruz	PLB	EC	‡ VW	+	0.1 2,4-D 0.01 BA; 1 g PH 1 g AC	Ishii *et al.* 1998
Phalaenopsis ***8**	Seedling leaf segment	PLB	Ka/Hy	-	2 g peptone	Amaki and Higuchi 1989 (Tanaka 1987: method)
Phalaenopsis ***11**	Callus	Callus	NP	+	TE, BH, AJ, PH	Ichihashi and Islam 1999
Phalaenopsis ***14**	PLB	Plant	Hy	-	2% sweet potato, 2.5% BH, 0.1% AC	Hsieh *et al.* 1997
Phalaenopsis ***16**	Seedling	Plant	Fir bark + perlite	-	DCPTA (*in vivo* treatments)	Keithly and Yokoyama 1990
Phalaenopsis Taisuko 339						Chan *et al.* 2003
Phalaenopsis To, T5, T10, Hikaru	Flower stalk bud	PLB	‡ VW→VW→ Hy	+	50 g AH, 50 g PH→2 g peptone	Chai *et al.* 2002
Phalaenopsis True Lady "B79-19"	Flower stalk bud	PLB	MS	+	1 NAA 3 BAP→ 0.2 NAA 1 BAP; 0.2% AC	Chen *et al.* 1998
	Leaf	PLB	VW	+	3 NAA 5 BAP	
Phalaenopsis Zada x Zada, Lipperose, Zauberrose	Leaf	PLB/plantlet	K	-	0.3 KNA 2 BAP; 100 g birch sap	Koch 1974; Zimmer and Pieper 1978
Pholidota chinensis	Leaf tip	PLB/plantlet	H, IY	-	0.2 NAA	Yam 1989; Yam and Weatherhead 1991a
Pholidota chinensis, *P. cantonensis*	Root tip	Shoot/plantlet	Kn^{m}	-	70 tCA	Yam 1989; Yam and Weatherhead 1991b
Phragmipedium Sedenii	Flower bud	PLB/plantlet	MS^{m}	-	0.1 NAA 10 BAP 40 AS	Fast 1979
Pleione sp.	Shoot tip	PLB/plantlet	K^{m}	-	3 g AC	Weatherhead and Harberd 1980
Pleione formosanum	Protocorm-derived callus	PLB/plantlet	‡ MS	-	5 2,4-D 0.5-1 TDZ	Lu 2004
Pogonia japonica	Rhizome tip	Plantlet	MS$_{L}$/B5$_{L}$/K^{m}/Hy^{m}	-	≥0.02 BA ≤0.2 NAA	Takahashi and Kondo 1998
Ponerorchis graminifolia	Embryo (immature seed)	Plantlet	MS/Hy	-	-	Nagashima 1989
Potinara sp.	Apical/axillary bud	Shoot/root	MS^{m}	+/-	0.1 NAA 10 AS 1 BA→0.1 NAA 30 AS 1 BA	Huang 1984
	Shoot tip	Plantlet	MS	-	0.1 NAA (+ computerization)	Tisserat and Vandercook 1986
Psygmorchis pusilla	Embryo (immature seed)	Plant	VW^{m} (Oxoid agar)	-	6% BH 0.1% AC	Vaz *et al.* 2004
Pterostylis sanguinea	Seed-derived protocorm	Shoot/tuber	OMA	-	5 µM JA	Debeljak *et al.* 2002
Renanetia	Floral bud	PLB	†§ MS→Hy→ MS	-	NH_4^+ vs NO_3^- effects	Kishi and Takagi 1997a
Renantanda Sanderi	Leaf	C/PLB	VW^{m}→VW	+	-	Goh and Tan 1982
	Shoot tip	PLB/plantlet	VW$_{L}$	+	-	Ghani *et al.* 1992a
	Shoot tip	PLB/plantlet	YII$_{L}$→ VW^{m}→VW	+	10% TJ 1.75 g peptone; 2.5 g Gaviota #67	Arditti and Ernst 1993
Renanthera imschootiana	Leaf base	PLB/Shoot/root	MPR	+	5 BA 2 NAA 2 g peptone BH→2 BA+NAA 1% AC	Seeni and Latha 1992
	Protocorm	Seedling	Ni	-	(Na-alginate synseed)	Chetia *et al.* 1998
Rhynchostylis gigantea	Shoot tip/stem bud	C/plantlet	Individual	+	0.1 NAA; 500 g tryptone	Vajrabhaya and Vajrabhaya 1970
	Shoot tip tTCL	Shoot/plant	MS	-	1 BAP 1 TDZ	Le *et al.* 1999
Rhynchostylis retusa	Leaf	C/plantlet	MPR^{m}→ MPR	-	1 NAA 1 K; 300 g peptone 2 g AC	Vij *et al.* 1984
	Root segment	PLB/plantlet	MPR/MS^{m}	-	1 NAA 1 K	Vij *et al.* 1987
	Embryo					Nath *et al.* 1991
Saccolabium calceolare	Inflorescence axis	PLB/plantlet	MPR^{m}	-	50 ml/l urea	Vij *et al.* 1986
Sarcanthus scolopendrifolius	Seedling	Plantlet	MS	-	10 BA 0.1 NAA	Lee *et al.* 1999
Schomburgkia superbiens	Vegetative shoot	C/PLB/plantlet	K^{m}$_{L}$→VW	+	0.1 K 2 IAA	Scully 1967
Sobralia macrantha	Seedling	Plantlet				Nishimura 1991
Sophrolaeliocattleya	Axillary bud	C/plantlet	MS^{m}	-	0.1 NAA→10 2,4-D 10 K	Kako 1969; Ishii *et al.* 1976

Table 1 (cont.)

Species, principal cultivar(s) + others	Explant source	Organ	Basal medium**	+/- CW	PGR/additive *1	Reference
Spathoglottis plicata	Root/seed	C/plantlet	VW			Beechy 1970
	Seedling hypocotyls	Root/shoot/ plant	W			Chennaveeraiah and Patil 1975
	Callus/seedling rhizome	C/root	MS^m	+	5 NAA 1 K→6 NAA 2 2,4-D 2 K→6 NAA 2 K	Bapat and Narayanaswamy 1977
	Flower bud	PLB	MS^m/K^m/ VW^m	+/-	2 IAA 2 K 2 AS/BA; BH	Lim-Ho *et al*. 1984
	Node/leaf/root	PLB/plantlet	MS^m	-	1 NAA 0.1 BA	Teng *et al*. 1997
	Protocorm	Protocorm	MS	-	10 ABA; sucrose 10%	Wang *et al*. 2003
Thunia alba	Flower stalk bud	PLB/plantlet	VW^m	+	100 tCA 10 BA→5 IBA→2 IBA 0.5 NAA→5 IBA	Singh and Prakash 1984
Vanda	PLB	Plantlet	VW^m/Hy	-	1 NAA 10 BA; 800 PVP 2 g AC	Tanaka *et al*. 1975
	Apical/axillary bud	PLB	VW/SH	+	0.25-0.5 NAA + BA	Khaw *et al*. 1978
	Root/leaf tip	PLB/plantlet	VW^m	-	1 IAA 0.1 2,4-D 0.1 BAP; 200 CH→0.1 IAA	Chaturvedi and Sharma 1986
	Flower stalk/bud	PLB/plantlet	K^m→VW	+	1 BA 1 K	Valmayor *et al*. 1986
Vanda coerulea (blue Vanda)	Leaf base Embryo	PLB/plantlet	MPR^m	+	1 NAA 1 BAP; 2 g peptone; 0.8 g Gaviota #63, BH	Mitra *et al*. 1976; Seeni 1988 Nath *et al*. 1991 Seeni and Latha 2000
	Shoot tip	PLB	VW	-	2 TDZ→2 IAA/3 IBA/NAA; 0.25 g peptone	Malabadi *et al*. 2004
Vanda cristata	Leaf					Sharma and Vij 1997
Vanda insignis X *V. tessellata*	Shoot tip	Plantlet	VW	+	-	Teo *et al*. 1973
Vanda spathulata	Stem node	Shoot	MPR	+	1 BA 1 IAA	Decruse *et al*. 2003a
	Leaf	Shoot	MPR	+	2:1 BA:IAA= 4-8 BA 5.7-28 IAA; 75 g BH	Decruse *et al*. 2003b
Vanda teres	Protocorm	Shoot	VW	+	1 BAP 0.5 NAA	Sinha and Roy 2004
Vanda (terete/strap leaf)	Stem	Shoot/root	VW^m	-	-	Sagawa and Seghal 1967
(terete leaf) Miss Joaquim	Shoot tip/axillary bud/root	Shoot/plantlet	W^m	+/-	-→2 2,4-DK→10% TJK→100 IAA	Goh 1970
(terete leaf) Miss Joaquim	Shoot tip	Plantlet	VW^m	+	-	Kunisaki *et al*. 1972
Vandofinetia	Flower stalk	PLB/plantlet	VW^m	+	-	Intuwong and Sagawa 1974
Vandofinetia Nara 'Yumika Pink'	Floral bud	PLB	†§ MS→Hy→ MS	-	NH_4^+ vs NO_3^- effects	Kishi and Takagi 1997a, 1997b
Vanilla planifolia	Nodal stem section	Shoot/root	MS^m	-	0.5 BAP; 1 g CH	Kononowicz and Janick 1984
	Aerial root tip	PLB/plantlet	K	-	1 NAA 0.1 K→1 2,4-D 0.1 K	Philip and Nainar 1986
	Lateral bud	C/shoot/root	LS^m	-	0.5 BAP 0.5 K; 1 g CH→0.2 NAA 0.1 BAP	Gu *et al*. 1987
	Callus					Davidson and Knorr 1991
	Axillary bud	Shoot	MS→MS_L	-	2 BA1 NAA→1 BA 0.5 NAA	George and Ravishankar 1997
	Shoot tip/node segment	Shoot/plantlet	V1-4 (N69; MS^m)	-	0.5 BAP; 0.05 d-biotin, 0.5 folic acid	Geetha and Shetty 2000
	Shoot tip/node	Shoot/root	MS	-	2 BA 1 NAA→0.01 BA 0.1 IBA; 40 µM $AgNO_3$	Giridhar *et al*. 2001
	Beans	SE	G	-	42 Cefotaxime sodium 33 Vancomycin-HCl	Podstolski *et al*. 2002
Vanilla walkeriae	Stem node	Shoot/root	MS	-	0.5 K 1 BA 1 g CH	Agrawal *et al*. 1992
Vascostylis Blue Fairy	Inflorescence	PLB/plantlet	VW	+	PH; BH	Intuwong and Sagawa 1973
Vuylstekeara Cambria	Shoot meristem	PLB/plantlet	† /K^m/VW^m	-	-	Morel 1960, 1970
	Plantlet	PLB/shoot/root	MS	-	0.2 NAA 0.2 BA; 1 g peptone	Kukułczanka *et al*. 1989
Zygopetalum	Shoot meristem	PLB/plantlet	† /K^m/VW^m	-	-	Morel 1960, 1970

Plant organ: C = callus, EC = embryogenic callus, PB = pseudobulb, PLB = protocorm-like body, SE = somatic embryo, TCL = thin cell layer; *1 = PGR/additive values in mgl^{-1}; *2 = plant material, culture vessels and conditions, media and procedures are the same as those followed for *Arachnis*, *Aranda* and *Aranthera* (Lim-Ho, 1981), ***3** = genotype-dependence found; ***4** = *Oncidium ampliatum*, *O. cebolleta*, *O.* Dr. Schragen, *O.* Golden Sunset Sunspot, *O.* Purple Envy, *O. sphacelatum*; ***5** = *Paphiopedillum lawrencianum* x *P.* Maudiae, *P. callosum*, *P. curtisii* Sanderae, *P.* Emerald x *P.* Alma Gevaert, *P. insigne* Harefield Hall, *P. villosum* x *P. insigne* Maulei, *P. nitens* Sallieri x *P. oenantum*, *P. callosum* x *P. lawreanceanum* var. *hyeanum*, *P. insigne* x *P. spicerianum*, *P. concobelatum* x *P. niveum*, *P.* Gwen Hannen x *P.* Phantasy; ***6** = *Phalaenopsis amabilis*, *P.* x Santa Cruz, *P.* Surfrider, *P.* Ruby Lips, *P.* Arcadia, *P. cochlearis*; ***7** = *Phalaenopsis* Münsterland Stern Alpha, Babette Symphony, Windspiel Düsseldorf, Barbara Moler Firecracker; ***8** = *Phalaenopsis* Surfrider x *P.* Joseph Hapton x *Doritaenopsis* cv. Kaala Gleam; ***9** = *Doritaenopsis* New Toyohashi #451, *D.* Hamakita Rainbow x *Phalaenopsis* Arai A-9; ***10** = 34 species of 15 genera (see text for details); ***11** = *Phalaenopsis* Hanaboushi, *P.* x Musashino, *P.* Snow Parade, *P.* Wedding Promenade, *P.* Hanaboushi x *P. equestris* Ilocos, *P.* (Grand City x Texas Thunder) x *P.* (Mikawa White x Wataboushi); ***12** = *Doritaenopsis* Su's Red Lip 'Ching Hua II' FCC/AOS x *D.* Amour Rojo 'Bonnie Vazquez'; ***13** = *Sophrolaeliocattleya* Jewel Box Scheherezade and *Brassolaeliocattleya* Rugley's Mill 'Mendenhall'; ***14** = *Phalaenopsis* Brother Mirage 'A79-69', *P.* True Lady 'A76-13', *P.* Asian Elegance 'B79-11', *P.* Taisuko Kaaladian 'F80-13'; ***15** = *Paphiopedilum* Connie, *P. barbatum*, *P. bullenianum*, *P. callosum*, *P. charlesworthii*, *P. ciliolare*, *P. hisutissimum*, *P. insigne*, *P. lawrenceanum*, *P. spiceranum*, *P. venustum*, *P. villosum*, 11 x *P.* hybrids; ***16** = *Phalaenopsis* X cv. #1609 [(Herbert Hager x Best Rose) x Snow Leopard], *P.* X cv. #12 (Capitola 'Moonlight' FCC/AOS x Joseph Hampton 'Diana' AM/AOS), *P.* X cv. #27 (Barbara Moler 'Gertie' AM/AOS x Zauberrose); ***17** = *Brassolaeliocattleya* Toshie Aoki 'Pizazz', Raye Holmes 'Mendenhall'; ***18** = *Phalaenopsis* Awayuki x Crescent, *Phal.* White Dream x Cassablanca Morning, *Phal.* (Grand City x Texas Thunder) x (Mikawa White x Wataboushi), *Phal.* Hatsuyuki x (Grand City x Texas Thunder). ** = Medium name, macro- or micro nutrient source. **Media**: B = Burgeff EG1 (Burgeff 1936), B5 = Gamborg B5 (Gamborg, Miller, Ojima, 1968), C = Churchill *et al*. (1970), Cu = Curtis (Curtis 1936; Arditti *et al*. 1982), G = Gamborg (Gamborg and Eveleigh 1968), H = Heller's (1953), HA = Harrison and Arditti (1970), H&A = Hoagland and Arnon (1950), Hy = Hyponex (Kano, 1965; Hyponex Japan, N:P:K = 6.5-6-19, NH_4-N:NO_3-N = 1:5.5), IY = Ichihashi and Yamashita (1977), K = Knudson C (1946), Ka = Kano (1965), Kn = Knop's (1984), Kyoto (Tsukamoto *et al*. 1963), L = Lindemann *et al*. (1970), LS = Linsmaier-Skoog (1965), MPR = Mitra-Prasad-Roychowdhury (Mitra *et al*. 1976), MS = Murashige and Skoog (1962), N = Norstog (1973), Ni = Nitsch (Nitsch and Nitsch 1969), NDM = new Dogashima medium (reference unclear), NP = new *Phalaenopsis* medium (Ichihashi 1992), OCM4 = Orchid culture medium 4 (Beardmore and Pegg 1981), OF = Ojima and Fujiwara (1962), OMA = Oatmeal agar (Clements and Ellyard 1979), RM = Reinert-Mohr (1967), SH = Schenk-Hildebrand (1972), T = Tsuchiya (Tsuchiya 1954; Wimber 1963), TGD = Thomale GD (1957),

Table 1 (cont.)

TT = Tsutsui and Tomita (Tomita and Tomita 1997), VW = Vacin and Went (1949), W = White (Singh and Krikorian 1981), Wi = Wimber (1963), WPM = Woody plant medium (reference unclear), YII = Yamada II (reference unclear); (medium) ^{m} = modified medium, ***A** = Wuxal (12:4:6), Kampsal (7:7:7), Hyponex (5:10:5), chemical compound fertilizer (18:18:18), M_L = liquid medium (subscript L); +/- CW = (with/without) coconut water; † = see Arditti and Ernst, 1993 for details of medium constituents. **Plant growth regulators**: 2iP = 6-(α,α-dimethylallylamino)-purine or 2-isopentenyl adenosine, 2,4-D = 2,4-dichlorophenoxyacetic acid, ABA = abscisic acid, AS = adenosine sulphate, BA = benzyladenine, BAP = 6-benzylaminopurine, DCPTA = 2-(3,4-dichlorophenoxy)triethylamine, IAA = 3-indole acetic acid, IBA = indole-3-butyric acid, GA = gibberellic acid (GA_3), JA = jasmonic acid, K = kinetin, KNA = potassium naphthylacetate, NAA = α-naphthalene acetic acid, Z = Zeatin, ZR = zeatin riboside. **Additives**: AC = activated charcoal, AH = apple homogenate, AJ = apple juice, BH = banana homogenate, CA = casamino acids, CH = casein hydrolysate, G = glycine, Gr = graphite (darkening agent), OCP = orthochlorophenoxyacetic acid, PH = potato homogenate, PVP = polyvinylpyrrolidone, TA = traumatic acid, *trans*-2-dodecene-1,12-dioic acid, tCA = *trans*-Cinnamic acid (anti-auxin), TIBA = 2,3,5-triiodobenzoic acid, TJ = tomato juice, YE = yeast extract; n.s. = not specified. In **all** cases (except for liquid cultures and ‡, which is Phytagel, Gellan gum or Gelrite®, a polymer of glucuronic acid, rhamnose, and glucose) the solidifying agent is agar, and under a 16-h photoperiod (otherwise specified in Arditti and Ernst, 1993, or marked as § if grown in the dark).
All medium additives reported as in original references but concentrations of PGRs converted to mgl^{-1}
Blank spaces indicate unclear, unspecified or unfound information

parts, designed to control morphogenesis more strictly than regular multi-tissue/organ explants (Teixeira da Silva 2003; reviewed in Teixeira da Silva 2013), especially from 6-7 week-old PLBs whose cells at this stage are highly meristematic (Prakash *et al*. 1996), used to hasten and improve plant regeneration has been reported in various orchids such as *Aranda*, *Cymbidium*, *Rhynchostylis* and *Spathoglottis* (Tran Thanh Van 1974a, 1974b; Begum *et al*. 1994b; Lakshmanan *et al*. 1995; Teng *et al*. 1997; Le *et al*. 1999). This is because of the availability of nutrients and growth promoting substances at the site of regeneration and due to the elimination of correlative control imposed by other tissues and organs.

Cells and protoplasts

Protoplasts have been successfully isoalted from *Cattleya*, *Cymbidium*, *Epidendrum*, *Ascocentrum*, and *Vanda* flower, root, and leaf tissue (Oshiro and Steinhart 1991). *Arachnis hypogea* mesophyll cells could divide and form PLBs (Joshi and Ball 1968), although it was Steward and Mapes (1971) who first successfully established *Cymbidium* plants from suspension cultures, and Singh (1987) from *Spathoglottis plicata* cells, it was Capesius and Meyer (1977) who successfully isolated nuclei from protoplasts. *Aerides*, *Acampe praemorsa*, *Agraecum giryamae*, *Brassavola*, *Cattleya*, *Geodorum densiflorum*, *Luisia zeylanica*, *Maxillaria tennuifolia*, *Oberonia santapui*, *Oncidium ampliatum*, *Paphiopedilum villosum*, *Phalaenopsis*, *Renantanda*, and *Vanda teres* protoplasts were isolated from young leaves or from protocorms, while those for *Dendrobium* and *Paphiopedilum* were isolated from other young plant parts (Teo and Neumann 1978a, 1978b; Price and Earle 1984; Seeni and Abraham 1986), those of *Bulbophyllum* and *Grammatophyllum elegans* from leaves and roots (Seeni and Abraham 1986), of *Cattleya* from protocorms, leaves and roots (Price and Earle 1984), from leaves and roots of *Epidendrum radicans* (Seeni and Abraham 1986), from protocorms in *Brassia maculata* and *Cymbidium pumilum* (Cepesius and Meyer 1977), while those of *Anoectochilus elatus*, *Cymbidium* Miracle x *Cymbidium* Alexanderi 'Golden Hill', *Calanthe discolor*, *C. masuca*, *Epidendrum*, and *Paphiopedilum insigne* were derived from leaf mesophyll (Yasugi *et al*. 1986; Gopalakrishnan and Seeni 1987). Despite protoplasts being isolated from *Dendrobium* 'Beach Girl' petals, *D.* 'Louis Bleriot' green leaves (Price and Earle 1984), *D. aggregatum* and *D.* 'Yukidaruma Queen' mesophyll (Yasugi *et al*. 1986), leaves and roots of *D. herbaceum* (Seeni and Abraham 1986), fusion and regeneration experiements were either difficult or not reported. Protoplasts from green sepals of *Angraecum giryamae* failed to form callus (Price and Earle 1984), but were used to make intrageneric hybridizations. Protoplasts have also been isolated from *Aranda* (Loh and Rao 1985), other orchids (reviewed in Arditti and Ernst 1993), and from *Barlia longibracteata*, used in intergeneric fusion experiments with *Ophrys lutea* and *O. bombylifera*, and for the study of endophyte/host interactions (Pais *et al*. 1983). Interspecific cell fusion of the bigeneric hybrid *Renantanda* (*Renanthera* × *Vanda*) with *Phalaenopsis* was achieved using young leaf protoplasts (Teo and Neumann 1978a, 1978b). Different basal media equally result in successful culture and proliferation of *Aranda* protoplasts (Arditti and Ernst 1993). Leaves, petals or roots could equally be used to derive protoplasts in *Arachnis* Maggie Oei (Arditti and Ernst 1993). Where orchids are used as parents for one or more intergeneric hybrids, as occurs with *Brassia*, protoplast isolation is useful as they can be used in fusion experiments involving genera used in sexual hybridization (Capesius and Meyer 1977). Aseptic cell cultures could be obtained first by the induction of callus on *Cymbidium* shoot tips, then transferring callus to a medium containing 2,4-D (Steward and Mapes 1971).

Cell ploidy is often due to endoreduplication, when one or several rounds of DNA synthesis occur in the absence of mitosis or cytokinesis resulting in an increase in the genomic DNA content, which appears to be extremely variable in the Orchidaceae, expecially in *Dendrobium* (Jones *et al*. 1998). Endoreduplication studies in *Phalaenopsis aphrodite*, *P. equestris*, and *Oncidium varicosum* showed that young floral buds had lower ploidy levels than older flowers, and that endoreduplication was a contributing factor to cell growth (Lee *et al*. 2004). PLB-propagated epiphytic *Cymbidium* hybrids and rhizome-propagated terrestrial *C. kanran* Makino demonstrated polysomaty from 2C to 16C, with roots and floral organs, excluding ovaries of hybrids, were highly polysomatic, as were the rhizomes and roots of *C. kanran* (Fukai *et al*. 2002), while Nagl (1972) claimed polysomaty in parenchyma cells. An increase in ploidy level was observed when 2,4-D or picloram were added to *Doritaenopsis* suspension cultures (Mishiba *et al*. 2001). High levels of polysomaty were also reported in *Phalaenopsis* (Mii *et al*. 1997) and *Dendrobium* roots (Jones and Kuehnle 1998). Endopolyploidy occurred in *Vanda* somatic tissues, induced more in germinating embryos by NAA than by GA_3, but observed only in the roots, leaves and the column, but not in the shoot apex, stem, perianth and pedicel (Lim and Loh 2003). Lin *et al*. (2001) estimated the nuclear DNA content of 18 *Phalaenopsis* species by flow cytometry and found that 2C values ranged from 2.74 to 16.61 pg, while Jones *et al*. (1998) showed that the range in *Dendrobium* was much narrower (1.73 to 4.23 pg per 2C). "Complement fractionation", a term describing the cell division products with variable chromosome numbers, usually found in polyploid orchids (Teoh 1982).

Root cultures, tubers, rhizomes

Cytokinin-induced shoot or rhizome formation in *Cymbidium* rhizome cultures is common (Ueda and Torikata 1968, 1969a, 1969b; Kokubu *et al*. 1980; Hasegawa *et al*. 1985; Lee and So 1985; Hasegawa and Goi 1987; Lee 1988a, 1988b; Shimasaki and Uemoto 1990). *Phalaenopsis* roots sometimes produce plantlets spontaneously in nature (Fowlie 1987), and can also be produced *in vitro* (Tanaka *et al*. 1976). Plantlets have also been produced from *Catasetum* (Kerbauy 1984b), *Rhynchostylis* (Vij *et al*. 1987) and *Cyrtopodium* (Sanchez 1988) root cultures. Plantlets were derived from the culture of *Bletilla striata* root tips (Yam and Weatherhead 1991b). Shimasaki and Uemoto (1987, 1990) demonstrated that the apical meristems of *Cymbidium kanran* and *C. goeringii* develop vigorous rhizomes *in vitro* at high NAA and 2,4-D concentrations; shoot formation from these rhizomes is accelerated when auxin:cytokinin is low; root formation occurred when both auxin and cytokinin were added, but only when potassium nitrate and ammonium nitrate were reduced. The conversion of root meristems into shoot cultures depends on the endogenous level of phytohormones in the explant, but appears to be induced strongly by the addition of IAA (Philip and Padikkala 1989). Using the *Mormodes histrio* method to culture roots 34 species of 15 genera shoots were obtained; within 24 species from *Catasetum* (10), *Clowesia* (2), *Cycnoches* (3), *Cyrtopodium* (1), *Galeandra* (3), and *Mormodes* (5) shoot formation could be induced in root tips; *Graphorkis lurida* and *Grobya galeata* produced only few PLBs (Hölters and Zimmer 1991). The quiescent center of *Spathoglottis* aerial roots showed low DNA synthetic activity relative to the rest of the cell population of the apical meristem (Raghavan and Goh 1995).

Rhizome regeneration to plants is the most important procedure in the propagation of terrestrial *Cymbidiums in vitro*. Generally BA has been used to initiate shoot buds from rhizomes (Lee *et al*. 1984; Chung *et al*. 1985a, 1985b; Hasegawa *et al*. 1985; Lee *et al*. 1986; Wang 1988; Shimasaki and Uemoto 1990; Paek and Yeung 1991; Choi *et al*. 1996), but this led to the formation of abnormal and hypertrophied shoot buds in *C. faberi* (Hasegawa *et al*. 1985). In general, terrestrial *Cymbidium* are usually derived from rhizomes originating from seeds germinated *in vitro*, isolated shoot meristems and flower buds cultured *in vitro* (Shimasaki and Uemoto 1991; Chang and Chang 1998). Plantlets needed a long time (10 months) to grow from *C. kanran* rhizomes (Lee *et al*. 1986), while plantlets derived from *C. forrestii* rhizomes showed retarded growth (Paek and Yeung 1991). Enhanced mitotic activity of root meristematic cells results in plant regeneration in *Catasetum* (Kerbauy 1984a; Vaz *et al*. 1998).

Tubers were formed *in vitro* when *Pterostylis sanguinea* seed-derived protocorms were placed on oatmeal agar in the presence of 5 μM jasmonic acid (Debeljak *et al*. 2002).

Leaf cultures

Even though early attempts at culturing leaf explants failed since cells were differentiated, the culture of mature differentiated palisade parenchyma was successful (Joshi and Ball 1968). Later on, however the formation of bulbils on leaf tips in a number of species was possible (Churchill *et al*. 1973) while restrepias could be propagated by leaf cuttings (Webb 1981). Juvenile leaves on protocorms are most likely to form PLBs, first produced in *Cymbidium* cultures of shoot meristems on a gyratory shaker in liquid medium (Wimber 1963). Since then, the formation of PLBs on leaf segments has been reported in *Aranda* (Fu 1979b), *Cattleya* (Champagnat *et al*. 1970), *Laeliocattleya* (Churchill *et al*. 1971, 1973; Ball *et al*. 1971), *Phalaenopsis* (Tanaka 1987), *Renantanda* (Goh and Tan 1982), *Rhynchostylis* (Vij *et al*. 1984), and *Vanda* (Tanaka *et al*. 1975). Leaf tips, especially mesophyll cells, were used to propagate *Epidendrum* and *Laeliocattleya* (Arditti *et al*. 1971), and leaf juvenility was shown to be an essential factor in the successful formation of callus from leaves (reviewed in Arditti and Ernst 1993). Both young (Loh *et al*. 1975) and old (Manorama *et al*. 1986; Fu 1978, 1979b) leaf tips could be used for the production of callus, PLBs and plantlets. The culture of young leaf bases was used to vegetatively multiply *Cattleya* (Champagnat *et al*. 1970), while leaf tips were used for clonal propagation (Churchill *et al*. 1971). Studies (Lavrentyeva 1986) on the clonal propagation of *Cymbidium* from leaf primordia concluded that: a) PLBs are larger when the cytokinin:auxin is 1:1 or 2:1, b) the optimal size of explants is about 0.5 mm including 3-4 leaf primordia, and c) PLBs arise at the base of leaf primordia. The effects of exogenous auxins and cytokinins (Chen and Chang 2001), tissue culture conditions, explant characteristics (Chen and Chang 2003b), aminocyclopropane-1-carboxylic acid (ACC) and ethylene inhibitors (Chen and Chang 2003a), auxin polar transport inhibitors (2,3,5-triiobenzoic acid, TIBA and 3,3′,4′,5,7-pentahydroxyflavone, quercetin) and an auxin antagonist (2-(*p*-chlorophenoxy)-2-methylpropionic acid, PCIB) (Chen and Chang 2004) on direct somatic embryogenesis were investigated in *Oncidium* leaf cultures. Somatic embryogenesis at the leaf tip, cut end and adaxial side of *Oncidium* 'Gower Ramsey' leaf explants was promoted by ancymidol and paclobutrazol, but inhibited by cycocel and GA_3, the former three being growth retardants (Chen and Chang 2003). The culture of *Phalaenopsis* leaf segments (Tanaka and Sakanishi 1980), in turn obtained from shoots derived from flower-stalk cuttings cultured *in vitro* (Tanaka and Sakanishi 1978), on gellan gum or Gelrite™ promoted the formation of callus-derived PLBs more than when agar was used as the medium solidifying agent (Ichihashi and Hiraiwa 1996; Ishii *et al*. 1998). Park *et al*. (2002) showed that the use of *Doritaenopsis* leaf TCLs could be used to generate more PLBs per explant than conventional explants.

Stems and shoot tips

Only *Arundina* stem segments or stem disks (Mitra 1971) and *Dendrobium* nodes have been cultured to date (Arditti *et al*. 1973). Shoots and roots of *Orchis maculata* were obtained from the culture of tuber buds (Thomale 1957) while the clonal propagation of virus-free *Anacamptis pyramidalis*, *Cymbidium*, *Miltonia*, *Odontoglossum*, and *Phaius* was achieved through the culture of virus-free apical meristems (Morel 1960, 1963, 1970). *Arunda* – a hybrid genus (*Arachnis* × *Vanda*) of considerable importance – shoot tips have been successfully cultured on both Vacin-Went and Schenk-Hildebrand media (Vajrabhaya and Vajrabhaya 1976; Cheah and Sagawa 1978; Sagawa and Kunisiaki 1982). The addition of NAA and 6-benzyladenine (BA) enhance the growth of PLB formation (Khaw *et al*. 1978), although BA was shown to be superior to NAA in *Cymbidium* (Matsui *et al*. 1970; Fujii *et al*. 1999a, 1999b). The use of stem nodes to induce new plantlets *in vitro* ensured the continuation of *Bletilla striata*, a very rare medicinal Chinese herbal medicine (Yam and Weatherhead 1991a). Arditti and Ernst (1993) claim that the methods used to clonally propagate *Brassavola*, *Brassocattleya*, *Brassolaelia*, *Cattleya*, *Laelia*, and *Laeliocattleya* can also be used for the trigeneric hybrid, *Brassolaeliocattleya*. Shoot cultures of *Cymbidium* produced more PLBs when in the presence of methyl jasmonate at <1 μM (Shimasaki *et al*. 2003a), while the addition of quartz porphyry "bakuhanseki" to ultrasonicated water increased the number of PLBs, shoots and roots in *Cymbidium eburneum*, *C. kanran* and *Phalaenopsis* (Shimasaki *et al*. 2003b). Putrescine stimulated PLB production in *Dendrobium* shoot tip cultures while spermidine and spermine inhibited PLB production (Saiprasad *et al*. 2004). Culture of *Dendrobium moschatum* stem discs was more successful in liquid culture (Kanjilal *et al*. 1999).

Flower buds, flower bud segments, and inflorescences

Dendrobium was micropropagated by using ovaries and immature seeds (Ito 1955, 1960), and has been similarly used to micropropagate a number of orchids, including *Vanilla*, *Phalaenopsis*, *Vanda*, and *Paphiopedilum* (reviewed in Arditti and Ernst 1993). The culture of *Ascofinetia*, *Neostylis*, and *Vascostylis* (Intuwong and Sagawa 1973), *Phalaenopsis*, *Phragmipedium* (Fast 1979) and *Cymbidium* (Kim and Kako 1984) were achieved by the use of young flower buds or inflorescences. When NAA and BA were used at 1 mg l^{-1} vegetative buds could be induced from gynostemia, ovaries and flower stalks, and the production was enhanced when liquid medium was used (Kim and Kako 1984). Flower stalk cuttings as explants for propagation has been used in *Epidendrum radicans* (Singh and Prakash 1982), *Thunia alba* (Singh and Prakash 1984), *Vanda*

Table 2 *In vitro* flowering studies in orchids (≤2000).

Plant species	Medium composition and pH	Other	Reference
Anacamptis (*Orchis*) *morio**		2½ years from seed to flower	Chia *et al*. 1999
Arethusa bulbosa	KCms + thiamine, niacin, charcoal phosphate buffer (pH 5.3)	Seedling	Yanetti 1996
*Barkeria barkeriola**	Solid	Seedling	Chia *et al*. 1999
Calypso bulbosa Aaron Island	Asymbiotic	Seedling	Ashmore 1995
Cymbidium (Maya-Ran, Shun-ran)	Solid	TC plantlet	Wang 1988a
Cymbidium ensifolium	W/MSs + 1/2 BA + 0.1/0.2 NAA	TC plantlet/seedling	Wang 1984 1988b
Cymbidium goeringii	MSs + 10% CW	TC plantlet	Wang 1988
Cymbidium nipponicum	Hy (pH 5.4)	Mycoparasitic; seedling	Mizuno *et al*. 1991
Cymbidium niveomarginatum	MS/Hy (pH 5.8)	Chinese terrestrial	Paek *et al*. 1989; Niimi *et al*. 1993
Dendrobium candidum	MSs + NAA, BA, polyamines	Flowers within 3-6 mo (20-30%)	Wang *et al*. 1990
Dendrobium Madame Thong-In*	Solid + BA	Seedlings	Chia *et al*. 1999
Dendrobium moniliforme	VW + 5 BA	Incomplete anther, stigma, petals	Duan and Yazawa 1994c
*Dendrobium secundum**	Solid (no treatment flower-induction)	Seedling flowering: no treatment	Chia *et al*. 1999
Dendrobium Sonia	Solid + BA?	Inflorescence explants	Goh 1996
Dendrobium (*striolatum* x *monophyllum*)*	Solid	Flowering within 12 months	Chia *et al*. 1999
Doriella Tiny	MSs/VWs/Hys + 5 BA + 15% CW + 2.5% sucrose (pH 5.6)	Little bud formation after 80 days	Duan and Yazawa 1994b, 1994c, 1995a
Doritis pulcherrima X *Kingiella philippinensis*	Hys + 5 BA + 15% CW + 2.5% sucrose (pH 5.6)	47% (40 days); 93% (80 days)	Duan and Yazawa 1994b
*Habenaria rhodocheila**	Solid	22 months to flowering	Chia *et al*. 1999
Laeliocattleya hybrid	½Ks (pH 4.9)	Seedling; 1st orchid *in vitro* flower	Knudson 1930
*Masdevallia floribunda**	Solid	Seedling	Chia *et al*. 1999
Oncidium sp., *O. ampliatum*, *O.* Golden Viper*	Solid	Seedling	Chia *et al*. 1999
Oncidium varicosum	Ks + 6% BH + 1% sucrose + 0.1% AC + 27.8 EDTA	"Microinflorescences"	Kerbauy 1984
Oncidium Baldim*	-	12 months to flowering from seed	Chia *et al*. 1999
*Orchis simia**	-	2½ years to flowering from seed	Chia *et al*. 1999
Phalaenopsis sp.	Solid	Flower stalk sections	Arditti and Ernst 1993
Phalaenopsis (White falcon X Persistent)	VW + 20% CW	Flower stalk section→flower stalk	Tanaka *et al*. 1988
Phalaenopsis Pink Leopard	VWs + BA (μM level) + varying levels of AC and/or N	36% (40 d); 70-71% (80-120 d)	Duan and Yazawa 1995b
Phalaenopsis Pink Leopard Petra	Hy + 5 BA	One-bud flower stalk node section	Duan and Yazawa 1994c
*Pleurothallis tuerckheimii**	Solid	Seedling	Chia *et al*. 1999
Psychopsis (*Oncidium*) *papilio**	Solid	Seedling	Chia *et al*. 1999
Psygomorchis (*Oncidium*) *pusilla**	Solid	Seedling	Livingston 1962; Chia *et al*. 1999
*Sarcochilus**	Solid	*In vitro* flowering "common"	Upton 1962
Serapias sp.*	Solid	Seedling	Chia *et al*. 1999
*Sphyrarhynchus schliebenii**	Solid	Seedling; 2 years to flower	Chia *et al*. 1999

Table is partially adapted from Table 1, Chia *et al*. (1999). * due to personal communication, the reference Chia *et al*. (1999) is used. AS = adenine sulphate; BM1 = van Waes and Debergh (1986), C = Curtis (Curtis 1936), Hy = Hyponex (Hyponex Japan, 6.5-6-19, N-P-K), Ka = Kano (1965), Mo = Mori (Mori *et al*. 1969), MT = Murashige and Tucker (1969), N = Norstog (1973), ND = New Dogashima, NN = Nitsch and Nitsch (1969), SH = Schenk-Hildebrand (1972), TGD = Thomale GD (1957), TT = Tsutsui and Tomita (Tomita and Tomita, 1997), W = White (1933). Superscripts: m = modified, s = solid, l = liquid; BH = banana homogenate, CW = coconut water, OMA = oatmeal agar, Mi = *myo*-inositol, PDA = potato dextrose agar, PJ = pineapple juice

(Sagawa and Sehgal 1967), and *Phalaenopsis* (Rotor 1949; Griesbach 1983; Tanaka 1992), but is not suitable for monopodial orchids.

In vitro flowering

The capacity to induce flowering of orchids *in vitro* has attracted considerable attention, mainly with the purpose of reducing the vegetative juvenile phase, which can range from 1-10 years, depending on the species and cultivar (Goh *et al*. 1982; Goh and Arditti 1985; Chia *et al*. 1999). Historically, the first orchid (*Laeliocattleya*) to flower *in vitro* was induced by Knudson in 1930. A number of reports are available on the induction of early flowering in orchids using tissue culture procedures (**Table 2**). *In vitro* flowering may be affected by stress (ABA-induced); complex additive addition such as banana homogenate; sugars, especially sucrose; nutrient concentration; flowering gradient; photoperiod; temperature effect, especialy vernalization; low pH; the presence of EDTA, a chelation agent; initial plant size and age; and the presence of PGRs (Chia *et al*. 1999). Even though surface-sterilized *Phalaenopsis* flower stalk nodes placed in Knudson C medium formed plants (Rotor 1949; **Table 1**) in the case of *Ascofinetia*, a monopodial orchid, the removal of buds would result in the loss of a plant so the tissue culture of inflorescences with flower primordia can result in flowering *in vitro*, or the production of PLBs or plantlets, depending on the culture medium (Intuwong and Sagawa 1973). It was found that the use of IAA conjugates, such as IAA-alanine or IAA-glycine substitute well as proliferation-inducing hormones (Griesbach 1983). *In vitro* flowering has been reported in *Cymbidium* (Wang and Xiong 1988; Paek *et al*. 1989; Niimi *et al*. 1993; Kostenyuk *et al*. 1999), *Dendrobium candidum* (Wang *et al*. 1993), *D. grex* Madame Thong-In (Yu and Goh 2000a), × *Doriella* (Duan and Yazawa 1994b), *Oncidium pusillum* (Livingston 1962), *O. varicosum* (Kerbauy GB 1984), *Phalaenopsis* (Duan and Yazawa 1995), and *Psygmorchis pusilla* (Vaz *et al*. 2004), the latter capable of developing inflorescences in the dark. *In vitro* flowering has been known to occur in *Phalaenopsis*, *Cymbidium*, *Arachnis* and *Oncidium* cultures when flower-stalk nodes have been used as the initial explant material (Intuwong *et al*. 1972; Arditti and Ernst 1993; Chia *et al*. 1999). BA, which has been shown to induce flowering *in vitro* in several plant species (Scorza 1982) has also been responsible for *in vitro* flower induction in several orchid genera when used in combination with NAA (Goh 1992, 1996), but resulted in abnormal flower bud formation in *Phalaenopsis* (Duan and Yazawa 1995). Flowering occurred in about 40 days and subcultured plantlets produced terminal flowers in 2-3 months when *Cymbidium ensifolium* mericlone PLBs were cultured on MS medium with 1 mg l^{-1} BA and 0.1 mg l^{-1} NAA (Wang *et al*. 1981, 1988; Wang 1988), while the same two PGRs could induce early flowering in *Dendrobium candidum* within 3-6 months from protocorms (Wang *et al*. 1990). High concentrations of sugar (glucose, sucrose or mannitol) also stimulated flowering in *Cymbidium ensifolium* (Wang *et al*. 1992) while the nitrogen content has been shown to influence flowering induction (Wada and Totsuka 1982; Tanaka 1986). A temperature ≤25°C is necessary for the emergence of a *Phalaenopsis amabilis* flower stalk from an intact plant, whether this be *in vitro* or *ex vitro* (Tanaka and Sakanishi 1978), and generally low temperatures are required for *Phalaenopsis in vivo* floral induction (Rotor 1952; Sakanishi *et al*. 1980) while high temperatures were shown to inhibit *in vitro* flowering (Chen *et al*. 1994), possibly as a result of low levels of endogenous gibberellins (Su *et al*. 2001), photoinhibition, or photodamage (He *et al*. 1998), but whose inhibition can be reversed by the application of GA_3 (Chen *et al*. 1994). The addition of spermine, or BA, or the combination of NAA and BA could induce protocorms or shoots to flower within 3-6 months with a 32-46% frequency, and to 83% when protocorms were pre-treated with abscisic acid (ABA) (Wang *et al*. 1997). The position of *Phalaenopsis* flower stalk nodes affected the regenerative outcome (Herrmann and Sell 1991). In *Doriella*, a 3-step process, namely a) the culture of floral stalk explants on a BA-supplemented Hyponex® medium for about 90 days, b) the culture of these shoots on BA-supplemented Vacin and Went medium for floral bud initiation, then c) floral development on BA-free Hyponex® medium (Duan and Yazawa 1994).

Somatic embryogenesis

PLBs are in fact somatic embryos (Steward and Mapes 1971; Begum *et al*. 1994; Ishii *et al*. 1998; Chen and Chang 2000; Teixeira da Silva and Tanaka 2006), with an intermediary callus sometimes being a pre-requisite for PLB formation (Colli and Kerbauy 1993; Chang and Chang 1998; Ishii *et al*. 1998). Secondary PLB formation and proliferation has been well documented (Tanaka and Sakanishi 1980; Griesbach 1983; Tanaka 1987; Amaki and Higuchi 1989). Hyperhydric *Doritaenopsis* PLBs, despite having a lower shoot-forming capacity than normal PLBs, had a greater capacity to form secondary PLBs (Zhou 1995). There are a variety of embryo developmental patterns in the Orchidaceae, *Cymbidium* being one variant in which there is an irregular pattern of early divisions and unique filamentous suspensor cells (Yeung *et al*. 1996; Huang *et al*. 1998). In *Spathoglottis plicata* embryo germination, cell divisions are confined to the proximal end of a protocorm whereas cells at the distal end undergo enlargement (Raghavan and Goh 1994). Somatic embryogenesis has been achieved in *Oncidium*, a sympodial orchid, through the use of flower stalk internodes (Chen and Chang 2000b) and in the absence of PGRs, whose presence cause, in part, somaclonal variation in orchid *in vitro* cultures (Tokuhara and Mii 1998). Embryogenic callus obtained from *Oncidium varicosum* was subcultured with difficulty and had a low regeneration capacity into PLBs (Lim Ho 1981). In contrast, embryogenic callus obtained from *Oncidium* 'Gower Ramsey' roots, greater than that of stem and leaf segments, could form somatic embryos that developed well into PLBs on hormone-free 1/2 MS and regenerated into healthy plantlets (Chen and Chang 2000b). The presence of thidiazuron (TDZ) was essential for the formation of somatic embryos when used alone in *Oncidium* (Chen *et al*. 1999), with 2,4-D in *Cymbidium* (Chang and Chang 1998) or *Paphiopedilum* (Lin *et al*. 2000). TDZ has also been shown to be important in increasing *Phalaenopsis* and *Doritaenopsis* protocorm proliferation rate and inducing PLBs (Ernst 1994), enhancing adventitious bud initiation (Chen and Piluek 1995), directing shoot regeneration from foliage (Nayak *et al*. 1997), and in combination with auxins, callus induction and maintenance (Chang and Chang 1998). The formation of embryogenic callus in *Dendrobium fimbriatum* can be induced on germinating or immature seeds in the absence of any PGRs (Roy and Banerjee 2003). The use of liquid medium was superior to solid medium in *Epidendrum radicans* embryo culture (Singh and Prakash 1985). Orchid embryos are most commonly cultured on solid media (Pierik 1987), or on media with glass or cotton wool for the support of seeds (Ernst 1975).

Embryo, ovule or green pod culture, the growing of ovules containing immature embryos excised from green fruits under aseptic conditions, was developed for *Spathoglottis plicata* (Sagawa and Valmayor 1966; Valmayor and Sagawa 1967; Lücke 1971a, 1971b; Sauleda 1976; Singh 1992).

SEED GERMINATION

Orchid seed germination *in vitro* dates back to the mid 19th C., which is possibly also the first plant to be propagated *in vitro* (Moore 1849). The first symbiotic germination of seeds *in vitro* was established in 1899 (Bernard 1899), mimicking mycorrhizal associations that occur naturally, whereas Lewis Knudson's axenic cultures were first used for the asymbiotic germination of seeds (Knudson 1922). Orchid seeds are minute and usually undifferentiated, and have unique characteristics quite unique from other Angiosperms (Arditti and Ghani 2000). Each contains an embryo composed of 8-100 cells with the endosperm underdeveloped or completely lacking (Wirth and Withner 1988). Often orchids begin the life cycle as saprophytes aided by mycorrhizae (Clements 1988; Arditti *et al*. 1990), which feed off the orchids' primary nutrient reserve, lipids (Manning and van Staden 1987). Many media have been used for the axenic culture of terrestrial and epiphytic orchid seeds (**Table 3**), with undefined organic supplements often being added to the media, including banana homogenate, potato extract, yeast, meat or fungal extract, dextrose, and peptone, among others. In general, *in vitro* germination of temperate terrestrial orchids is more difficult than with tropical epiphytic orchids (de Pauw *et al*. 1995), the former usualy requiring a low salt medium, with Harvais (1982) claiming the importance of cytokinins for terrestrial orchid germination. Symbiotic seed germination is greatly influenced by the type of fungus, temperature and light, although contradictory results exist with respect to light, many advocating the necessity of dark incubation (Rasmussen 1995; Ronconi 1998) while others show the stimulation of seed germination by light (Rasmussen *et al*. 1990b; Zettler and McInnis 1994). Light-enhanced germination of *Goodyera repens* is controlled by the red part of the light spectrum (McKinley and Camper 1997), but darkness stimulated symbiotic seed germination in *Spiranthes odorata* (Zettler and Hofer 1997). The starting pH of orchid seedling media is mildly acidic (5.2-0.5), but the acidity increases as a result of plantlet growth (Ernst *et al*. 1992). The choice of medium has an impact on the success of seed germination, whose period is species dependent (Yam and Weatherhead 1988); incidentally the species within this latter study were not included in **Table 3** since too many variables are unknown. Snow (1985, 1987) demonstrated how the simple inclusion of 0.1% hydrogen peroxide, catalase and/or other biocidal agents into Knudson's C medium allowed for the successful germination of seed of at least 16 genera.

In vitro seed germination is a powerful tool to preserve rare, native, and often overcollected orchid species, producing large numbers while maintaining a more variable gene pool than through clonal micropropagation, as proved with *Encyclia boothiana* (Stenberg and Kane 1998). In modern conservation studies, as those conducted on *Cypripedium reginae*, *C. parviflorum* and *Platanthera grandiflora*, seed pretreatment with 10% sodium hypochlorite (a surface sterilant often used to enhance germination of terrestrial species) followed by infection with the appropriate symbiotic fungus, i.e. *in vitro* bioassay germination test is preferred to the conventional chemical procedures of staining with triphenyl tetrazolium chloride or acid fuschin (Vujanovic *et al*. 2000). Knudson, Vacin and Went, and MS media were all suitable for germination ans subsequent growth of *Epidendrum* seedlings, being an epiphytic orchid with a preference for low salt concentration for optimal growth (Sagawa and Valmayor 1966; Valmayor and Sagawa

Table 3 *In vitro* seed germination studies (≤2004); for more generalized review, see Kane *et al*. (2008).

Plant species	Symbiont(s)	Medium composition**	Chilling	Germination efficiency (%)	Other	Reference
Aceras anthropophorum	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<5.7	Surface-sterilized	Van Waes and Debergh 1986
Actanthus reniformis	None	K + 15% CW	n.s.	n.s.	Supplementary lighting	McIntyre *et al*. 1974
Amerorchis rotundifolia	*Rhizoctonia*, *Ceratobasidium*, *Thanatephorus*	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few (no percentage)	Sterilized; North America	Smreciu and Currah 1989
Anacamptis pyramidalis	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<89.8	Surface-sterilized	Van Waes and Debergh 1986
Anoectochilus formosanus	None	½MS (+ 100 *myo*-inositol) +0.2% AC +8% BH	n.s.	78.1	Surface-sterilized	Shiau *et al*. 2002
	None	½MS_L + 2 BA	n.s.	86.7	Sterilized; 4 months	Nalawade *et al*. 2003
	None or *Rhizoctonia* R01, R02, R04	OMA (symbiotic); MS (asymbiotic)	n.s.	0-79.5	Surface-sterilized	Chou and Chang 2004
Aplectrum hyemale	None	N; initial sterilization; dark incubation	n.s.	25 (in 37 days)	To plantlet stage	Henrich *et al*. 1981
	None or *Ceratobasidium* sp., *Tulasnella* sp.	C	4 °C (over $CaCl_2$)	1-10	Surface-sterilized	Oliva and Arditti 1984
Arundina chinensis	*Epulorhiza* isolates	PDA/OMA + 3 rose bengal + 50 streptomycin	None	n.s.	RAPD/ITS analyses	Shan *et al*. 2002
Bletia urbana	None	K/MS + CW + 3% sucrose	n.s.	21-100	Habitat re-introduction	Rubluo *et al*. 1989
Bletilla striata	None	K+0.5/1.5% glucose, sucrose, trehalose, mannitol	22 °C (17 weeks)	n.s.	Surface-sterilized; dark	Smith 1973
	None	Hy/MS/KC + 2% sucrose	n.s.	0-100	Surface-sterilized	Nagashima 1982a
	Rhizoctonia repens	Hy/K + 2 g peptone	None	0-93.4	Protocorm enhanced	Masuhara and Katsuya 1989
	None	Kyoto/Hy/MS – Fe-EDTA; CH 0.035 GA_3	1 °C (4 months)	0-22	Light effects	Ichihashi 1990
	None	Hy/$Kyoto^m$	n.s.	0-100	Different sterilization	Yanagawa *et al*. 1995
	None	ND→PVS2	0 °C (3 h)	60	Vitrification method	Ishikawa *et al*. 1997
	None	ND	n.s.	0-98.7	Dehydration 2 weeks	Hirano *et al*. 2005
Brassocattleya Deesse x *Cattleya* Mount Shasta	None	K + surfactants (on glasswool platform)	24 °C ± 2	2.5-100	18-h photoperiod	Ernst and Arditti 1984
Bratonia (*Miltonia flavescens* x *Brassia longissima*)		K^m→MS/Kn + 5 BA				Popova *et al*. 2003
Caladenia spp. x 4	None	K + 15% CW	n.s.	n.s.	Supplementary lighting	McIntyre *et al*. 1974
Calanthe discolor	None	Hy/MS/KC + 2% sucrose	n.s.	0-100	Surface-sterilized	Nagashima 1982a
	None	MS^m/Kn; 1 NAA 0.2 BA; 3 g G; 1 g ethephon	5 °C	10 (control); 60 (exper.)	Sonication	Miyoshi and Mii 1988 1995
Calanthe sieboldii	None	MS/MS+SH/Hy + 1 putrescine or 25 AS	None	n.s.	Immature seeds	Park *et al*. 2000
Calochilus robertsonii	None	K + 15% CW	n.s.	n.s.	Supplementary lighting	McIntyre *et al*. 1974
Calopogon tuberosus	None	N; initial sterilization; dark incubation	n.s.	25 (in 29 days)	To plantlet stage	Henrich *et al*. 1981
	None	C/K/N/WS+1 BA/0.25 BAP,Z/1-5 K/0.1 NAA	4 °C (over $CaCl_2$)	30-100	Sterilization; organics	Arditti *et al*. 1985
Calypso bulbosa	None	C/K/N/WS+1 BA/0.25 BAP,Z/1-5 K/0.1 NAA	4 °C (over $CaCl_2$)	50-80	Sterilization; organics	Arditti *et al*. 1981 1985
	None	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few (no percentage)	Sterilized; North America	Smreciu and Currah 1989
Cattleya aurantiaca	None	K/K^m	5 °C (≤ 6 months)	n.s.	Store in $CaCl_2$	Harrison 1977; Harrison and Arditti 1970 1978
	None	K + 20-50 Ethephon	None	≤65	Reduced leaves	Tamanaha *et al*. 1979; Ernst *et al*. 1992
	None	K + a range (25) of bactericides and fungicides	n.s.	n.s.	Phytotoxicity tests	Thurston *et al*. 1979
	None	K	22 °C ± 2	n.s.	Developmental study	Nishimura 1981
	None	K + 20-50 Ethephon; range of phytoalexins	None	≤65	Phytotoxicity	Hills *et al*. 1984
Cattleya (*aclandiae* x *schoeffeldiana*) x *aclandiae*	*Tulasnella asymetrica*	BSB + 0.02 YE	n.s.	Growth measurements	Surface-sterilized; light	Beyrle and Smith 1993a
Cattleya loddigesii	None	Hy/$Kyoto^m$	n.s.	0-100	Different sterilization	Yanagawa *et al*. 1995
Cephalanthera rubra	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<62.6	Surface-sterilized	Van Waes and Debergh 1986

Table 3 (cont.)

Plant species	**Symbiont(s)**	**Medium composition****	**Chilling**	**Germination efficiency (%)**	**Other**	**Reference**
Chiloglottis gunnii	None	K + 15% CW	n.s.	n.s.	Supplementary lighting	McIntyre *et al*. 1974
Coeloglossum viride	*Rhizoctonia*, *Ceratobasidium*, *Thanatephorus*, *Sistotrema*	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few-many (no percentage)	Sterilized; North America	Smreciu and Currah 1989
Corallorhiza trifida						Zelmer and Currah 1995
Cryptostylis erecta	None	K + 15% CW	n.s.	n.s.	Supplementary lighting	McIntyre *et al*. 1974
Cymbidium Hawtescens x *C. lowianum*	None	K + 10% CW	4 °C	100	Surface-sterilized	Dalla Rosa and Laneri 1977
Cymbidium sp.						Paek *et al*. 1997 1989
	None	VW 2.5 folic acid 1 NAA 20 tryptophan	None	85		Bannerjee and Mandal 1999
Cymbidium aloifolium	None	PM/VW^{m}/MS/KC^{m}	n.s.		Elongation with PGRs	Gupta and Bhadra 1998
Cymbidium goeringii	None	MS/Hy	n.s.	0.1-60	Surface-sterilized	Nagashima 1982b
Cymbidium kanran Makino	None	K/MS + 1 NAA 0.1 K	None	36-44	3% H_2O_2	Kokubu *et al*. 1980
	None	Ka^{m}: Hy + 2 g peptone	None	n.s.	Total darkness	Shimasaki and Uemoto 1990
	None	TT + 1 NAA (or MT +1 NAA or Hy + 1 GA_3)	None	≤93.9	J- and F-type proembryos	Nagashima 1998
Cypripedium acaule	None	MS + 1 K + 0.1 NAA; 19 g dextrose; 1.2 PH	25 °C	4-73	Surface-sterilized; dark	St-Arnaud *et al*. 1992; Lauzer *et al*. 1994
Cypripedium calceolus	None	N; initial sterilization; dark incubation	n.s.	1 (in 46 days)	To plantlet stage	Henrich *et al*. 1981
	None	TGD^{m} ± 0.2 BA	6 °C (4-8 weeks)	<38.3	Surface-sterilized	Van Waes and Debergh 1986
	None	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few (no percentage)	Sterilized; North America	Smreciu and Currah 1989
var. *pubescens*		MS/MS_1 + 10% CW	5 °C (8 weeks)	80-90	Mature capsules	Chu and Mudge 1994
	None	BM_1 + CH	20 °C ± 2	40	Ethanol-flamed; dark	Wagner and Hansel 1994
Cyp. acaule, *C. calceolus*, *C. californicum* +3	None or *Ceratobasidium* sp., *Tulasnella* sp.	C/Hy/N/OMA	4 °C (over $CaCl_2$)	1-80	Dark/light	Oliva and Arditti 1984; Arditti *et al*. 1985
Cypripedium candidum, *C. californicum*, *C. reginae*	None	N; initial sterilization; dark incubation	n.s.	1-25 (in 45-81 days)	To plantlet stage	Henrich *et al*. 1981
Cypripedium candidum, *C. reginae*, *C. calceolus*	None	N^{m} + 0.8 BA/2iP	4 °C (2 months)	~94	BA delays rooting	Harvais 1982; de Pauw *et al*. 1992 1995
Cyp. debile, *C. henryi*, *C. japonicum*, *C. tibeticum*	None	MS PGR-free (gelrite)	n.s.	0.5-65	Surface-sterilized	Hoshi *et al*. 1994
Cypripedium macranthos	None	NaOCl (0.5% Cl) + $Ca(ClO)_2$ (3.2% Cl)	4 °C (2 months)	58-70		Miyoshi and Mii 1998
Cypripedium reginae	None	Bu/FN/K	5 °C	≤93	Surface-sterilized	Ballard 1987
Dactylorchis purpurella	None	K+0.5/1.5% glucose, sucrose, trehalose, mannitol	22 (17 weeks)	n.s.	Surface-sterilized; dark	Smith 1973
Dactylorhiza fuchsii	None	K/N/MS + 3% sucrose	2-26 (≤126 days)	10-74	Surface-sterilized; dark	Pritchard *et al*. 1999
Dactylorhiza incarnata	None	TGD^{m} ± 0.2 BA	6 °C (4-8 weeks)	<93.2	Surface-sterilized	Van Waes and Debergh 1986
	Rhizoctonia				Low N concentration	Beyrle *et al*. 1991
	Epulorhiza repens; *Ceratorhiza* spp.	0.1% yeast extract 1% sucrose 0.9% cellulose	5 °C	≤6.25	Darkness germinated	Dijk and Eck 1995
Dactylorhiza maculata	None	TGD^{m} ± 0.2 BA	6 °C (4-8 weeks)	<88.6	Surface-sterilized	Van Waes and Debergh 1986
Dactylorhiza maculata var. *maculata*, *D. sambucina*	*Rhizoctonia*, *Ceratobasidium*, *Thanatephorus*, *Sistotrema*	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few-many (no percentage)	Sterilized; European	Smreciu and Currah 1989
Dactylorhiza majalis	None	TGD^{m} ± 0.2 BA	6 °C (4-8 weeks)	<78.3	Surface-sterilized	Van Waes and Debergh 1986
	Rhizoctonia	OMA	20 °C (6 weeks)	8-100	Surface-sterilized	Rasmussen *et al*. 1989 1990a; Rasmussen 1990
	Epulorhiza repens; *Ceratorhiza* spp.	0.1% YE 1% sucrose 0.9% cellulose	5 °C	≤10.4	Darkness germinated	Dijk and Eck 1995
	None	K/N/MS + 3% sucrose	2-26 °C (≤126 d)	28-93	Surface-sterilized; dark	Pritchard *et al*. 1999
Dactylorhiza praetermissa var. *junialis*	None	TGD^{m} ± 0.2 BA	6 °C (4-8 weeks)	<91.6	Surface-sterilized	Van Waes and Debergh 1986
	Epulorhiza repens; *Ceratorhiza* spp.	0.1% YE 1% sucrose 0.9% cellulose	5 °C	≤10.4	Darkness germinated	Dijk and Eck 1995
Dactylorhiza purpurella	*Ceratobasidium* spp., *Thanetephorus* sp., *Rhizoctonia* sp.	OCM + 0.2 Kinetin	22-24	n.s.	Darkness	Beardmore and Pegg 1981

Table 3 (cont.)

Plant species	Symbiont(s)	Medium composition**	Chilling	Germination efficiency (%)	Other	Reference
Dactylorhiza sambucina	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<80.3	Surface-sterilized	Van Waes and Debergh 1986
Dendrobium (*ionoglossum* x spp.)	None	$Ch/Y/Me/G/K/VW/G^m$	n.s.	100	Surface-sterilized	Mowe 1973
Dendrobium anosmum	None	K/N/MS + 3% sucrose	2-26 (≤126 days)	96-99	Surface-sterilized; dark	Pritchard *et al*. 1999
Dendrobium candidum		MS + BH PH; *Quercus glauca* bark			Alcoholic extract	Liu *et al*. 1988
Dendrobium crumenatum						Vellupillai *et al*. 1997
Dendrobium huoshanense		§ MS/K + 1 BA 0.5 NAA				Yang *et al*. 1989
Dendrobium kingianum	None	$Hy/Kyoto^m$	n.s.	0-100	Different sterilization	Yanagawa *et al*. 1995
Dendrobium linawianum						Chen *et al*. 1995
Dendrobium lindleyi		MS + 2 NAA 1 IBA 1 K; 0.02% AC				Kaur and Sarma 1997
Dendrobium tosaense	None	½MS/MS 3% sucrose→MS 8% BH/PJ/CW				Tsay *et al*. 2004
Dendrobium transparens	None	Hy/MS/OKF1/KC	n.s.	58-78	Native; Bangladesh	Alam *et al*. 2002
Dendrobium Jaquelyn Thomas White		‡ VW^m→‡ MS + CW				Kuehnle and Sugii 1992
Dendrobium Lady Hamilton	None	VW_L→VW	n.s.	n.s.	Air flow system	Cheng and Chua 1980
Dendrobium Yukidamma King	None	$Hy/Kyoto^m$	n.s.	0-100	Different sterilization	Yanagawa *et al*. 1995
Dipodium sp.	None	K + 15% CW	n.s.	n.s.	Supplementary lighting	McIntyre *et al*. 1974
Disa spp. x 7 (summer rainfall)	None (seed testa vital)	K + 0.2% AC; Half-MS + 0.2% AC 0.01% Mi	25 °C ± 2	n.s.	NaOCl; constant light	Thompson *et al*. 2001
Diuris laxiflora	yes	Paclobutrazol	None			Hollick *et al*. 2002
Diuris longifolia	None or *D. longifolia* wild fungi	‡ OMA/Agrosoke/perlite/*Allocasuarina* mulch	16 °C ± 2	≤50	Surface-sterilized; dark	Oddie *et al*. 1994
Diuris punctata +1	None	K + 15% CW	n.s.	n.s.	Supplementary lighting	McIntyre *et al*. 1974
Diuris punctata	*Tulasnella calospora*	2.5% OMA; 20% CW; 1% cellulose	2 °C	10	Surface-sterilized; dark	Clements and Ellyard 1979
Doritis pulcherrima						Kanchit 2000
Elythranthera brunonis	None or *E. brunonis* wild fungi	‡ OMA/Agrosoke/perlite/*Allocasuarina* mulch	16 °C ± 2	≤50	Surface-sterilized; dark	Oddie *et al*. 1994
Encyclia boothiana var. *erythronioides*	None	K^m/MS	None	29-54.2	For conservation	Stenberg and Kane 1998
Encyclia tampensis	*Epidendrum conopseum* mycorrhizal fungus, *Epulorhiza*	2.5% OMA; (effect of bark substrates*)	6 °C (24 months)	≤94.1	Surface-sterilized; dark	Frei 1973*; Zettler *et al*. 1999
Epidendrum fulgens	None	VW	n.s.	n.s.	*Chl* A/cytokinin analysis	Mercier and Kerbauy 1991
Epidendrum radicans O'brienianum	*Rhizoctonia* (from *Cymbidium*)	VW/K + various vitamins	n.s.	n.s.	Surface-sterilized	Vacin and Went 1949; Nair 1982 Hijner and Arditti 1973
Epipactis atrorubens	None	C/K/N/WS+1 BA/0.25 BAP,Z/1-5 K/0.1 NAA	4 °C (over $CaCl_2$)	<1	Sterilization; organics	Arditti *et al*. 1981 1982a
	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<71.9	Surface-sterilized	Van Waes and Debergh 1986
Epipactis helleborine	None	$C/N/C^m$	n.s.	<75%	Mature/immature seed	Arditti *et al*. 1982a
	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<26.8	Surface-sterilized	Van Waes and Debergh 1986
Epipactis gigantea	None	C/K/N/WS+1 BA/0.25 BAP,Z/1-5 K/0.1 NAA	4 °C (over $CaCl_2$)	<1-80	Sterilization; organics	Arditti *et al*. 1981 1982a
	None	N; initial sterilization; dark incubation	n.s.	100 (in 38 days)	To plantlet stage	Henrich *et al*. 1981
Epipactis palustris	*Rhizoctonia*, *Sistotrema*	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few (no percentage)	Sterilized; European	Smreciu and Currah 1989
	None/*Rhizoctonia*	K/VW/MB/OMA	4-8 °C (8-12 wks)	1-50	Seasonality	Rasmussen 1992
Eriochilus cucullatus	None	K + 15% CW	n.s.	n.s.	Supplementary lighting	McIntyre *et al*. 1974
Erythrorchis ochobiensis	*Lyophyllum shimeji*, *Tricholoma fulvocastaneum*	Mo + 2 YE + 2% glucose	25 °C (1 week)	0-most	Amorphous profiles	Umata 1997
	E. crocicreas, *Ganoderma*, *Loweporus*, *Microporus*, *Phellinus*	Mo + 2 YE + 2% D-mannitol	25 °C (3 weeks)	100	EtOH, CaOHCl	Umata 1998

Table 3 (cont.)

Plant species	**Symbiont(s)**	**Medium composition****	**Chilling**	**Germination efficiency (%)**	**Other**	**Reference**
Eulophia spp. x 6	*Cytosporella*, *Basidiomycete*, *Rhizoctonia*, *Mortierella* spp.	2% OMA + 10 g BH 0.2 g AC				Ochora *et al*. 2001
Eulophia gonychila	None	K/N/MS + 3% sucrose	2-26 (≤126 days)	76-100	Surface-sterilized; dark	Pritchard *et al*. 1999
Geodorum densiflorum	None	PM/MS	n.s.		Elongation: IAA/BAP	Bhadra and Hossain 2003
Glossodia major	None	K + 15% CW	n.s.	n.s.	Supplementary lighting	McIntyre *et al*. 1974
Goodyera macrophylla	None	K^m	None	100	Darkness germinated	Fernandes *et al*. 1999
Goodyera oblongifolia	None	C/K/N/WS+1 BA/0.25 BAP,Z/1-5 K/0.1 NAA	4 °C (over $CaCl_2$)	0-90	Sterilization; organics	Arditti *et al*. 1981 1982b
	None	N; initial sterilization; dark incubation	n.s.	100 (in 38 days)	To plantlet stage	Henrich *et al*. 1981
Goodyera pubescens						Zettler and McInnis 1993
Goodyera repens	*Rhizoctonia goodyerae-repentis*	PDA/Pfeffer 1% cellulose agar+fungicide: TBZ	4 °C	n.s.	6 weeks darkness	Alexander and Hadley 1984; Hadley 1984
	Rhizoctonia, *Ceratobasidium*, *Sistotrema*	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few-many (no percentage)	Sterilized; North America	Smreciu and Currah 1989
Goodyera repens var. *ophioides*	None	PDA	None	33	Effect of action spectra	McKinley and Camper 1997
Goodyera tesselata	None	C/K/N/WS+1 BA/0.25 BAP,Z/1-5 K/0.1 NAA	4 °C (over $CaCl_2$)	20	Sterilization; organics	Arditti *et al*. 1981, 1982b
Gymnadenia conopsea	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<81.3	Surface-sterilized	Van Waes and Debergh 1986
	Rhizoctonia, *Ceratobasidium*, *Thanatephorus*, *Sistotrema*	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few (no percentage)	Sterilized; European	Smreciu and Currah 1989
Gymnadenia odoratissima	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<64.1	Surface-sterilized	Van Waes and Debergh 1986
	Rhizoctonia, *Ceratobasidium*, *Thanatephorus*, *Sistotrema*	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few (no percentage)	Sterilized; European	Smreciu and Currah 1989
Habenaria dentata	*Ceratorhiza* isolates					Shan *et al*. 2002
Habenaria radiata	None	Hy/$Kyoto^m$	n.s.	0-100	Different sterilization	Yanagawa *et al*. 1995
Haemaria discolor	None or *Rhizoctonia* R01, R02, R04	OMA (symbiotic); MS (asymbiotic)	n.s.	0-80.9	Surface-sterilized	Chou and Chang 2004
Haemaria discolor var. *dawsoniana*	*Rhizoctonia* isolates	MS/Hy 1/2/3; OMA; 2% sucrose	None	34-63	Inoculation of seedlings	Chang and Chou 2001
Limodorum abortivum	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<2.7	Surface-sterilized	Van Waes and Debergh 1986
Liparis loeselii	None	N; initial sterilization; dark incubation	n.s.	25 (in 37 days)	To plantlet stage	Henrich *et al*. 1981
Liparis nervosa	*Rhizoctonia repens* #624	Cellulose/inulin/pectin/ mannitol/galactose	None	n.s.	Effect of C-source	Tsutsui and Tomita 1990
Listera ovata	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<38.4	Surface-sterilized	Van Waes and Debergh 1986
	Epulorhiza sp.	PDA	20 °C (42 d)	21.5	Surface-sterilized; dark	Rasmussen *et al*. 1991
Microtis media	Yes	Paclobutrazol	None			Hollick *et al*. 2002
Microtis spp. x 5	None	K + 15% CW	n.s.	n.s.	Supplementary lighting	McIntyre *et al*. 1974
Neottia nidus-avis	*Rhizoctonia*, *Ceratobasidium*,	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few (no percentage)	Sterilized; European	Smreciu and Currah 1989
	Sebacina spp.	*Fagus salvatica* woodland (UK + Germany)	n.s.	n.s.	ITS-RFLP	McKendrick *et al*. 2002
Nigritella nigra	*Rhizoctonia*, *Ceratobasidium*, *Thanatephorus*, *Sistotrema*	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few (no percentage)	Sterilized; European	Smreciu and Currah 1989
Ophrys apifera, *O. spegodes*	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<44.6-63.7	Surface-sterilized	Van Waes and Debergh 1986
Ophrys lutea, *O. fusca*, *O. speculum*	None	C^m	None	≤60	(Im)mature seeds	Barroso *et al*. 1990
Ophrys sphegodes						Mead and Bulard 1975
Ophrys x 13	None	M^m 10% CW 10% PJ 0.05% AC	4 °C (3 days)	45-85	(Im)mature seeds	Kitsaki *et al*. (2004)
Orchis coriophora, *O. mascula*, *O. morio*	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<31.9-87.4	Surface-sterilized	Van Waes and Debergh 1986
Orchis fuchsii, *O.masculata*	None	N; initial sterilization; dark incubation	n.s.	50-100 (in 34-77 days)	To plantlet stage	Henrich *et al*. 1981
Orchis laxiflora						Mead and Bulard 1975 Ozkoc and Dalci 1994

Table 3 (cont.)

Plant species	Symbiont(s)	Medium composition**	Chilling	Germination efficiency (%)	Other	Reference
Orchis morio	*Rhizoctonia*, *Ceratobasidium*, *Thanatephorus*, *Sistotrema*	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few-many (no percentage)	Sterilized; European	Smreciu and Currah 1989
	Ceratobasidium	OMA/rock wool + 1% sucrose	n.s.	n.s.	Surface-sterilized	Beyrle and Smith 1993b
	Epulorhiza repens; *Ceratorhiza* spp.	0.1% YE 1% sucrose 0.9% cellulose	5 °C	≤8.5	Darkness germinated	Dijk and Eck 1995
Paphiopedilum sp.	None	$Tsu^{m}L$ + colchicine	n.s.	n.s.	Polyploid induction	Watrous and Wimber 1988
Paphiopedilum callosum, *P.* x 2 hybrids	None	Bu/K/N/TGD	4 °C	0-100	Sterilized; dark/light	Stimart and Ascher 1981
Paphiopedilum ciliolare	None	TGD^{m}	n.s.	29-86	Sterilized 8-mo capsules	Pierik *et al.* 1988
Paphiopedilum insigne var. *sanderae*	None	MS/Hy	n.s.	0.1-60	Surface-sterilized	Nagashima 1982b
Paphiopedilum rothschildianum	None	K/N/MS + 3% sucrose	2-26 (≤126 days)	26-37	Surface-sterilized; dark	Pritchard *et al.* 1999
Paphiopedilum sukhakulii + hybrids		§ N/B				Tay *et al.* 1988
Paphiopedilum Dorama x *P.* Sully	None	K + 10% CW	4 °C	100	Surface-sterilized	Dalla Rosa and Laneri 1977
Paphiopedilum Souvenir x *P.* Cameo +4	None	TGD + 2% sucrose	n.s.	n.s.	Surface-sterilized	Flamée 1978
Paphiopedilum Winston Churchill x *P.* Evansrose	None	TGD^{m} + 0.2% Nuchar C (AC) + 5% BH	22-27 °C (200 d)	n.s.	Water-rinsed only	Ernst 1974
Phaius tankervilliae	None	NN + CW/PJ/BH (2%)	None	70	170 d old seeds	Bhuyan and Deka 1999
Phalaenopsis amboinensis +1	None	K/TGD + 0.2% ; pH 5.4-5.6	n.s.	n.s.	Aseptic removal: capsule	Ernst 1975
Phalaenopsis lueddemanniana	None	K	22 °C ± 2	n.s.	Developmental study	Nishimura 1981
Phalaenopsis hybrid	None	K→MS/B5 + 2.5% sucrose 7% BH 2 g AC	None	n.s.	96% EtOH surface sterile	Hinnen *et al.* 1989
Phalaenopsis hybrids	None	$Hy/Kyoto^{m}$	n.s.	0-100	Different sterilization	Yanagawa *et al.* 1995
Phalaenopsis Habsburg, *P.* Ruth Burton	None	K + 0.4% multi ologosaccharide	n.s.	n.s.	Surface-sterilized	Ernst and Arditti 1990
Phalaenopsis Polka x *P.* Opaline	None	K + 10% CW + 0.2% AC + BH	4 °C	100	Surface-sterilized	Dalla Rosa and Laneri 1977
Piperia elegans var. *elata*, *P. maritima*	None	C/K/N/WS+1 BA/0.25 BAP,Z/1-5 K/0.1 NAA	4 °C (over $CaCl_2$)	1	Sterilization; organics	Arditti *et al.* 1981, 1985
Polystachya spp.		2% OMA + 10 g BH 0.2 g AC				Ochora *et al.* 2001
Platanthera bifolia, *P. chlorantha*	None	TGD^{m} ± 0.2 BA	6 °C (4-8 weeks)	<67.9-91.6	Surface-sterilized	Van Waes and Debergh 1986
Plat. dilatata, *P. stricta*, *P. flava*, *P. hyperborea*	None	N; initial sterilization; dark incubation	n.s.	1-50 (in 34-88 days)	To plantlet stage	Henrich *et al.* 1981
Platanthera hyperborea, *P. saccata*	None	C/K/N/WS+1 BA/0.25 BAP,Z/1-5 K/0.1 NAA	4 °C (over $CaCl_2$)	<1-80	Sterilization; organics	Arditti *et al.* 1981, 1985
Plat. bifolia, *P. hyperborea*, *P. obtusata*, *P. orbiculata*	*Rhizoctonia*, *Ceratobasidium*, *Thanatephorus*, *Sistotrema*	FAST (asymbiotic), WCA (symbiotic)	1-6 °C	Few-many (no percentage)	Sterilized; U.S.A./Europe	Smreciu and Currah 1989
Platanthera integrilabia	16 fungal endophytes; best = *P. ciliaris* endophyte	OMA	4 temperatures	73.1	Endangered terrestrial	Zettler and McInnis 1992 1994
Platanthera praeclara	*Ceratorhiza* sp., *Epulorhiza* sp.	PDA	5 °C (4 months)	≤34.6 (of ≤36.5 viability)	95% EtOH + 5% NaOCl	Sharma *et al.* 2003
Polystachya spp. x 6	*Fungi imperfecti* (?), *Ceratobasidium* sp.	2% OMA + 10 g BH 0.2 g AC	18-22 °C	n.s.	Surface-sterilized	Ochora *et al.* 2001
Ponerorchis graminifolia	None	Kyoto/Hy/MS – Fe-EDTA; CH 0.035 GA_3	1 °C (4 months)	0-22	Surface-sterilized	Ichihashi 1989
	Rhizoctonia repens	Hy/K + 2 g peptone	None	8.8-26.8	Protocorm enhanced	Masuhara and Katsuya 1989
Pterostylis spp. x 10	*Ceratobasidium cornigerum*	2.5% OMA; 20% CW; 1% cellulose	2 °C	100	Surface-sterilized; dark	Clements and Ellyard 1979
Pterostylis spp. x 7	None	K + 15% CW	n.s.	n.s.	Supplementary lighting	McIntyre *et al.* 1974
Pterostylis sanguinea	Yes	Paclobutrazol	None			Hollick *et al.* 2002
Pterostylis vittata	*Pseudomonas putida*, *Xanthomonas maltophilia*, *Bacillus cereus*	KB; IAA (enhance) GA3 (inhibit) K (suppress)	None	n.s.	30 d dark→20/25 d light	Wilkinson *et al.* 1989, 1994
Serapias vomeracea var. *laxiflora*						Ozkoc and Dalci 1993
Spathoglottis plicata	None	W + 2% sucrose + 10% CW + 1 IAA + 1 Kin	n.s.	n.s.	60% humidity; callus	Chennaveeraiah and Patil 1975
	Rhizoctonia AM9	2.4% potato dextrose	None	84 (infection)	Encapsulated seeds	Tan *et al.* 1998
	None	MS + 10% CW + 2% sucrose; 10 ABA	None	n.s.	Dehydrin by Western	Wang *et al.* 2002, 2003

Table 3 (cont.)

Plant species	Symbiont(s)	Medium composition**	Chilling	Germination efficiency (%)	Other	Reference
Spathoglottis pubescens	*Epulorhiza* isolates	PDA/OMA + 3 rose bengal + 50 streptomycin	None	n.s.	RAPD/ITS analyses	Shan *et al*. 2002
Spiranthes cernua						Zettler and McInnis 1993
Spiranthes gracilis, *S. romanzoffiana*	None or *Ceratobasidium* sp., *Tulasnella* sp.	C/Hy/N/OMA	4 °C (over $CaCl_2$)	20-30	Dark/light	Oliva and Arditti 1984
Spiranthes hongkongensis	*Epulorhiza* isolates	PDA/OMA + 3 rose bengal + 50 streptomycin	None	n.s.	RAPD/ITS analyses	Shan *et al*. 2002
Spiranthes odorata	*Epulorhiza* isolates	2% OMA	-7 °C (32 months)	11-29.2	Surface-sterilized; dark	Zettler and Hofer 1997
Spiranthes romanzoffiana	None	N; initial sterilization; dark incubation	n.s.	75 (in 38 days)	To plantlet stage	Henrich *et al*. 1981
Spiranthes sinensis	*Rhizoctonia anastomosis*, *R. solani*, *R. repens*	Hy + 2 g peptone	None	47.5-94.6	Protocorm enhanced	Masuhara and Katsuya 1989 1994
	Rhizoctonia repens #624, #706	Cellulose/inulin/pectin/ mannitol/galactose	None	n.s.	Effect of C-source	Tsutsui and Tomita 1990
	Ceratobasidium cornigerum	0.3 % OMA				Uetake and Peterson 1997/8; Uetake *et al*. 1997
Spiranthes spiralis	None	TGD^m ± 0.2 BA	6 °C (4-8 weeks)	<82.5	Surface-sterilized	Van Waes and Debergh 1986
Thelymitra sp.	*Tulasnella calospora*	2.5% OMA; 20% CW; 1% cellulose	2 °C	15	Surface-sterilized; dark	Clements and Ellyard 1979
Thelymitra spp. x 9	None	K + 15% CW	n.s.	n.s.	Supplementary lighting	McIntyre *et al*. 1974
Vanda (terete/strap leaf) Miss Joaquim, TMA	None	Individual + 2iP; VW/MPR	n.s.	n.s.	Surface-sterilized; dark	Mathews and Rao 1980 1985
Vanda teres	None	VW + 2% sucrose 1 BAP 0.5 NAA 2 peptone	n.s.		Indigenous Bangladesh	Sinha and Roy 2004
Zeuxine strateumatica	None	MS/W/K + 1 IAA + 1 K + 20% CW	n.s.	n.s.	Surface-sterilized	Arekal and Karanth 1978

** = Medium name, macro- or micro nutrient source. M_L = liquid medium (subscript L); AS = adenine sulphate; BM_1 = van Waes and Debergh (1986), BSB = Burgeff Sb (Burgeff, 1936), Bu = Burgeff EG1 (Richter, 1972), C = Curtis (Curtis, 1936), Ch = Chang's (Mowe, 1973), FN = Fast (Fast, 1974), G = Graeflinger (Mowe, 1973), Hy = Hyponex (Hyponex Japan, 6.5-6-19), Ka = Kano (1965), KB = King's B (King *et al*. 1954), KC = Knudson C (Knudson, 1949), Kyoto (Tsukamoto *et al*. 1963), M = Malmgren (1996), MB = Mead and Bulard (1975), Me = Meyer (Mowe, 1973), Mo = Mori (Mori *et al*. 1969), MPR = Mitra-Prasad-Roychowdhury (Mitra *et al*. 1976), MT = Murashige and Tucker (1969), N = Norstog (1973), ND = New Dogashima, NN = Nitsch and Nitsch (1969), OCM = orchid culture medium (Bearmore and Pegg, 1981), OKF1 = Alam *et al*. (2002), PM = Phytamax, SH = Schenk-Hildebrand (1972), TGD = Thomale GD (1957), Tsu = Tsuchiya (Wimber, 1963), TT = Tsutsui and Tomita (Tomita and Tomita, 1997), Y = Yamada (Mowe, 1973), W = White's (White, 1933), WS (Wolter and Skoog, 1966); BH = banana homogenate, CW = coconut water, OMA = oatmeal agar, Mi = *myo*-inositol, PDA = potato dextrose agar, PJ = pineapple juice, YE = yeast extract
All medium additives reported as in original references but concentrations of PGRs converted to mgl^{-1}; blank spaces indicate unclear, unspecified or unfound information

1967). The use of NAA at 1 mg l^{-1} enhanced *Epidendrum radicans* seed germination and subsequent growth (Nair 1982), but at higher concentrations it stimulated callusing; similar effects were observed in *Cypripedium* (Boesmann 1962) and *Vanda* (Mathew and Rao 1980). Banana homogenate was shown to promote seed germination in *Phalaenopsis* (Hinnen *et al*. 1989).

It was found that the use of immature *Cymbidium* seeds, obtained from capsules 80-90 days following anthesis could be successfully germinated *in vitro* (Bannerjee and Mandal 1999), circumventing the poor germination of mature seeds associated with dormancy. The use of Norstog medium (Norstog 1973) was superior to Harvais, Van Waes and Debergh media when germinating *Cypripedium* seeds *in vitro* (de Pauw and Remphrey 1993). Seed and medium prechilling and liquid suspension cultures were favourable conditions for 80% seed germination in *Cypripedium calceolus* (Chu and Mudge 1994). Gelrite proved to be more suitable than agar while Agrosoke, ultrafine perlite or *Allocasuarina* leaf mulch could be used as an *in vitro* substrate for *Elythranthera brunonis* or *Diuris longifolia* seed germination (Oddie *et al*. 1994). Vaccuum infiltration of van Waes and DeBergh medium into *Cypripedium* seeds increased germination, even after lignification of the seed coat (Wagner and Hansel 1994). Nagashima (1994) could successfully germinate 47 species from within 22 genera on Hyponex® medium and continue their development into protocorms and rhizomes; the highest germination rate (0.8%-100%, depending on the species) was obtained when embryos were at least at the octant stage (Nagashima 1993). Often the successful germination of orchid seeds *in vitro* is hampered by the infection of the medium by fungi and bacteria, and a number of fungicides and bactericides were successfully applied to the *in vitro* seed germination of 11 orchid species (Brown *et al*. 1982).

CONVENTIONAL BREEDING AND GENETICS

Molecular methods are increasingly popular to resolve the complex phylogenetic relationships in the Orchidaceae. Initial studies employed DNA sequences from orchids that took advantage of loci from the plastid genome and were focused at the family and subfamily levels (Qamaruz-Zaman *et al*. 1998; Cameron 2004 and references therein). Efficient DNA (Lim *et al*. 1998) and RNA extraction procedures that overcome the presence of carbohydrates, phenolics, and other compounds that bind to and/or co-precipitate with RNA, have been devised (Knapp and Chandlee 1996; Champagne and Kuehnle 2000). Chromosome analysis and karyological studies (D'Emerico *et al*. 1992), such as mitotic and meiotic counts revealed the smallest chromosome number in the Orchidaceae, in *Psygomorchis pusilla*, n=5 (Pessoa and Guerra 1999), the existence of polyploidy and aneuploidy in *Ophrys orchis* and *Anacamptis* (Bianco *et al*. 1991), the difference between 8 orchid species in Bangladesh (Sheikh *et al*. 1993), sterility barriers in hybrids (Stort 1984), polyploidy in *Phragmipedium* (Wimber 1983), population studies of *Habenaria* (Felix and Guerra 1998), and variation and polyploidy (Chase *et al*. 1988) in *Oncidium* and allied genera, intersectional and intergeneric hybrids (Charanasri *et al*. 1973; Charanasri and Kamemoto 1975; Phang *et al*. 1979, 1981), *Dendrobium* (Wilfret *et al*. 1979) and the Vandeae (Arends and Van der Laan 1986). Colchicine treatment (125 µg l^{-1}) can be used to induce tetraploids in *Doritis pulcherrima* with a 44-46% conversion efficiency (Hsieh *et al.* 1991), or in *Paphiopedilum* (Watrous and Wimber 1988), while colchicine-induced amphidiploidy is essential for successful seed-propagated *Dendrobium* breeding (Amore and Kamemoto 1992; McConnell and Kamemoto 1993). Karyotypic analyses of *Phalaenopsis* and *Doritis* species revealed that the differential accumulation of constitutive heterochromatin is the causal agent for karyotype variation (Kao *et al.* 2001). Floral fragrance "fingerprinting" also provides an extremely sensitive and specific profile (e.g. Bergström *et al.* 1992).

Methylated C-glycosylflavones, the major constituents of most Ornithocephalinae members, can also be used as taxonomic markers (Williams *et al.* 1994).

Internal transcribed spacer (ITS)

In order to understand the relationships within orchid subtribes and genera, the nuclear ITS region (often complemented with sequences from the plasmid *trnL-F* or *matK* region) has become popular, although studies exist that have used DNA sequences from the nuclear and mitochondrial genomes (e.g. Freudenstein and Chase 2001; Soliva *et al.* 2001; Bateman *et al.* 2003). Nuclear rDNA ITS were used to elucidate the taxonomic relationships of nearly 100 slipper orchid (Cypripedioideae) species (Cox *et al.* 1997), while fungal ITS RFLP analysis and sequence of the ITS and nuclear LSU ribosomal gene fragments allowed Taylor *et al.* (2003) to understand the evolutionary dynamic associations between orchids and their mycorrhizal fungi, van den Berg *et al.* (2000) to establish phylogenetic relashionships wthin the Laeliinae, McKendrick *et al.* (2002) to understand symbiotic germination and spacial development in a field trial, and Tsai *et al.* (2004) to determine the genetic relationship of *Dendrobium* species in Taiwan. Recent studies identified the homologue of *LEAFY* (*LFY*) – a central regulatory gene in *Arabidopsis thaliana* flower development control – from *Orchis italica*, *OrcLFY* (Montieri *et al.* 2004), which served to construct molecular phylogenies. rDNA ITS were used to study the genetic relatedness between Sardinian and Sicilian *Orchis*, *Anacamptis* and *Neotinea* endemics (Cafasso *et al.* 2001a), and rRNA ITS to phylogenetically place mycorrhizal isolates in the *Rhizoctonia solani* species complex and determining host specificity (Pope and Carter 2001). rDNA ITS were used to authenticate medicinal *Dendrobium* species, which are rather expensive and in which adulteration is frequent; this is required to protect consumers since the low (1%) intraspecific variation among the species alows the 2 ITS regions to be adopted as a molecular marker for differentiating medicinal *Dendrobium* species, and to support conservation measures (Lau *et al.* 2001). 18S-26S ITS, *matK*, *trnT-L*, *trnL-F*, *rpL* 16 analyses allowed for the monotypic genus, *Kitigorchis*, to be reappraised (Yukawa *et al.* 2003). Lineages of tropical orchids were identified through sequencing of the nrDNA ITS (Otero *et al.* 2002). AT-rich satellite DNA from *Cymbidium* protocorm nuclei could be isolated by complexing DNA with Ag+ in a Ag+/CS_2SO_4 density gradient centrifugation (Capesius 1976).

Isozymes and allozymes

Isozymes have also been used to study orchid gene variation, gene flow and genetic drift in orchid populations (Rossi *et al.* 1992; Tremblay and Ackerman 2001), identification of new wild *Epipactis* hybrids (Harris and Abbott 1997), interspecific relationships in ten European orchids (Schlegel *et al.* 1989), introgression in *Cypripedium* (Klier *et al.* 1991), *Cymbidium* (Obara-Okeyo *et al.* 1998), ecologically extensive and morphologically variable *Cypripedium* spp. (Klier *et al.* 1991; Case 1993, 1994), AFLP-confirmed polymorphic *Dactylorhiza* (Hedrén *et al.* 1996a, 1996b, 1996c), Danish and other *Epipactis* (Scacchi *et al.* 1987; Ehlers and Pedersen 2000), *Gymnaedenia conopsea* (Scacchi and de Angelis 1989), early and late-flowering populations of *Gymnadenia conopsea* (Soliva and Widmer 1999), Lepanthes (Carromero *et al.* 1998), and *Orchis* (Scacchi *et al.* 1990; Corrias *et al.* 1991). High genetic diversity, both at a population and species level, was found in *Pteropstylis aff. picta* using 16 enzyme loci (Sharma *et al.* 2003), while isozyme analysis using 4 enzyme systems (GPI, UDP, ME, LAP) allowed for the clear identification of inter-specific hybrids (Sharma and Jones 1999). Despite the use of 8 enzyme systems for 70 *Cymbidium* cultivars, no cultivar showed a unique pattern, but the TPI (triosephosphate isomerase) system gave one "diagnostic" pattern (Obara-Okeyo *et al.* 1997), while isozyme variation for *in vitro*-cultured PLBs were obtained for AAT-1 and PGM-1 loci (Obara-Okeyo and Kako 1997). Protein electrophoresis was employed to assess the polyploid origin and to determine genetic variability within and among populations of *Spiranthes diluvialis*, an orchid whose polyploidy maintains genetic variation in allopolyploid species (Arft and Ranker 1998). Similar studies confirmed high genetic variability (69% polymorphism in 16 isozme loci) in Australian *Pterostylis gibbosa* populations (Sharma *et al.* 2000, 2001, 2003). Allozyme loci and cpDNA (*trn*L(UAA) intron) analyses were used to identify the hybrid nature of *Dactylorhiza insularis* (Bullini *et al.* 2001), while cultivar and species distinction in *Phalaenopsis* Blume cultivars was possible through allozyme analysis (Hsieh *et al.* 1992). Allozyme analysis of 3 populations of *Cypripedium fasciculatum* show low allozyme diversity suggesting that populations may depend on asexual propagation for recruitment, directly affecting conservation management practices (Aagaard *et al.* 1999). Allozyme analysis indicated that gene flow in *Gymnadenia conopsea* early- and late-flowering populations was low, caused by a difference in flowering phenology, and only four cpDNA haplotypes were found that differed only in the number of microsatelite repeats (Soliva and Widmer 1999). In order to confirm the polyploid nature of Oncidiinae species, isozyme numbers were compared: diploid and aneuploid species exhibit the same number of isozymes, whereas allopolyploids have more (Chase and Olmstead 1988).

AFLP, DAF, RFLP and RAPD

Genetic variation at a tissue, hybrid and cultivar level were measured using amplified fragment length polymorphisms (AFLPs) in vandaceous orchids (Chen X *et al.* 1999) and *Dendrobium* (Xiang *et al.* 2003). The use of AFLPs is useful since intensive breeding activities of tyropical orchids have given rise to many hybrids, among which genetic relationships are difficult to evaluate due to free interbreeding of different species in the same genus or even from different genera (Hedrén *et al.* 2001), the use of hybrids for further breeding, use of abbreviated or trade names and sometimes international non-disclosure of parentage for commercial purposes (Hong and Chuah 2003).

Random amplified polymorphic DNA (RAPDs; 100 primers tested) and isozyme analysis (aspartate aminotransferase and phosphoglucomutase) were used to assess the somaclonal variation derived from *Phalaenopsis in vitro* cultures (Chen *et al.* 1998), while RAPDs using 15 10-mer arbitrary primers allowed a total of 132 RAPD markers, 78% of which were polymorphic, to distinguish 36 *Cymbidium* cultivars (Obara-Okeyo and Kako 1998), which verified results obtained through isozyme analysis (Obara-Okeyo and Kako 1997). Incidentally, somaclonal variation found at low levels in *Vandofinetia* was attributed to the problem of hyprhydricity (Kishi *et al.* 1997). Similar studies showed the phylogenetic relationships between Korean *Cymbidium goeringii* and other *Cymbidium* cultivars (Choi *et al.* 1998), while these relationships in the genus *Calanthe* were discerned using RAPDs and isozymes (Hyun *et al.* 1999). Polymorphism was detected in *Cattleya* (Benner *et al.* 1995) and *Paphiopedillum* (Min and Tan 1996) using RAPDs, while RAPDs also allowed for the establishment of phylogenetic relationships in *Cattleya* and allied genera (Jin *et al*. 2004a, 2004b). RAPDs were also used to identify Oncidiinae cultivars (Tsai and Huang 2001), one of the most cytologically, ecologically and morphologically diverse groups of the orchid family: 14 10-mer primers revealed 263 bands, of which 257 were polymorphic (Tsai *et al*. 2002). Population studies determining intra- and interpopulation gene flow of 3 populations totalling 52 plants of *Epidendrum conopseum* were possible by identifying 11 polymorphic bands using 7 random primers (Bush *et al*. 1999). Italian *Ophrys* taxa were also differentiated using 11 different 10-mer primers (Grünanger *et al*. 2002). RAPDs and allozyme markers were used to study the levels of genetic variation and patterns of population structure of *Eulophia sinensis*, *Spiranthes hongkongensis*, *Zeuxine gracilis*, and *Z. strateumatica*, and in order to develop suitable conservation strategies for these wild orchids (Sun 1997; Sun and Wong 2001). Both RAPD and isozyme analyses were used to evaluate the genetic structure of natural populations of *Goodyera procera* (Wong and Sun 1998), and to distinguish somaclonal variants in *Phalaenopsis* tissue culture (Chen WH *et al*. 1998, Chen YC *et al*. 2000). PAGE-silver staining was 5 times more sensitive in identifying molecular markers in 6 *Phalaenopsis* species than agarose gel electrophoresis-EtBr staining (Fu *et al*. 1994).

Restriction fragment length polymorphism (RFLP), yielding different fragment sizes following chloroplast digestion with *DraI* followed by hybridization with an *rbcL* probe, was used to analyze the mode of inheritance of chloroplasts in both interspecific hybrids of *Phalaenopsis* and intergeneric hybrids of *Phalaenopsis* and *Doritis* (Chang *et al*. 2000). Cladistic parsimony analyses of *rbcL* nucleotide sequence data from 171 taxa representing narly all tribes and subtribes of the Orchidaceae were conducted (Albert 1994; Cameron *et al*. 1999). The choloroplast *ndhF* gene was used to determine phylogenetic distances between members of the Epidendroideae (Neyland and Urbatsch 1996) while the chloroplast genome was probed for restriction fragment variation in the Dendrobieae (Yukawa *et al*. 1993) and the Oncidiinae (Chase and Palmer 1989). Consensus multiplex PCR-RFLP, the simultaneous amplification of two or more target DNA sequences in the same reaction, was developed and employed to detect mitochondrial (mt)DNA variation in three orchid species, *Spiranthes hongkonggensis*, *S. sinensis*, and *S. spiralis* (Sun 1996; Chen and Sun 1998). Chloroplast DNA-RFLP was used to identify the hybrid origin of *Orchiaceras bergonii* (Cozzolino and Aceto 1994). DNA amplification fingerprinting (DAF) in *Phalaenopsis* showed that a 165-330 µg DNA per g fresh weight could be applied to a polyacrylamide gel electrophoresis-silver stain 3-5-fold more sensitive than agarose gel electrophoresis-EtBr stain and just 1 µl sample was needed (Fu *et al*. 1994).

Microsatellites

Microsatellite primers (6) were isolated for *Gymnadenia conopsea* (Gustaffson and Thoren 2001; Campbell *et al*. 2002), and 6 for *Serapias vomeracea*, which could be used a sprimers for population genetic studies (Pellegrino *et al*. 2001), and in one study the genetic pattern was studies in 5 microsatellite loci within 5 Swedish populations of the rare orchid, *G. odoratissima* (Gustaffson and Sjogren 2002). Another study used these microsatellites to demonstrate a drastic genetic divergence and significant habitat differentiation between early- and late-flowering variants of plants morphologically belonging to *G. conopsea* (Gustaffson and Lonn 2003). A minisatellite tandem repeat locus was found in the chloroplast genome of *Orchis palustris*, serving as a valuable marker for orchid population genetic studies (Cafasso *et al*. 2001b). Microsatellite loci were isolated from *Ophrys araneola* in order to understand the influence of sexual deception on genetic population structure and to estimate gene flow (Soliva *et al*. 2000).

FLOWERING CONTROL AND FLORAL DEVELOPMENT

Floral development in orchids at the molecular level has begun (Yu and Goh 2001) where it was found that the orchid floral homeotic gene of *Dendrobium DOMADS1*, is a marker gene specifically expressed in the transitional shoot apical meristem during floral transition (Yu *et al*. 2002). An AP3-like MADS gene (*OMADS3*) in *Oncidium* Gower Ramsey was shown to regulate floral formation and initiation (Hsu and Yang 2002), or *DOMADS1,2,3* in *Dendrobium grex* (Yu and Goh 2000a, 2000b), and when *OMADS1* is ectopically expressed in *Arabidopsis thaliana*, flowering time genes (Teixeira da Silva and Nhut 2003) were upregulated and plants flowered early (Hsu *et al*. 2003). In addition, there was homeotic conversion of sepals into carpel-like structures and p*etal*s into staminoid structures. Twenty-one *DwMYB* genes (transcription regulators) from *Dendrobium* Woo Leng were isolated, characterized, and their expression pattern examined during flower development (Wu *et al*. 2003). cDNAs representing genes expressed in a stage-specific manner during ovule development have been isolated from *Phalaenopsis* SM 9108 (Wang *et al*. 1999). An *Aranda* Deborah MADS box gene, *om1* was discovered after screening with an *Arabidopsis* agamous cDNA probe (Lu *et al*. 1993).

The limit that angiosperms have in producing flower colours is due to the lack of an anthocyanin biosynthetic gene or the substrate specificity of a key anthocynanin biosynthetic enzyme, dihydroflavonol 4-reductase (DFR), as occurs in *Cymbidium* hybrida flowers, which primarily produce cyanidin-type (pink to red) anthocyanins and lack the pelargonidin-type (ornage to brick red) anthocyanins (Johnson *et al*. 1999). Anthocyanins have been used to elucidate taxonomic and phylogenetic relationships in ten European orchids (Strack *et al*. 1989). A strong correlation exists between pH and floral favonoids in *Dendrobium* spp. (Kuehnle *et al*. 1997). cDNA clones encoding chalcone synthase (CHS), a key enzyme involved in flavonoid and anthocyanin biosynthesis were isolated from a cDNA library constructed from flowers of the orchid, *Bromheadia finlaysoniana*; RT-PCR analysis demonstrated that CHS transcripts could be amplified from all parts of the plant and floral organs (Liew *et al*. 1998a, 1998b). cDNA clones of dihydroflavonol 4-reductase (DFR), which catalyzes the NADPH-dependent conversion of dihydroflavonols into unstable flavan-3,4-diols, the intermediate precursors for the anthocyanins were also isolated from *B. finlaysoniana* (Liew *et al*. 1998a, 1998b).

A novel cytokinin oxidase, *DSCKX1* (*Dendrobium* Sonia cytokinin oxidase), was found by mRNA differential display from shoot apices of *Dendrobium* Sonia cultured in the presence of BA; transgenic orchid plants overexpressing *DSCKX1* showed reduced levels of cytokinins and subsequently exhibited slow shoot growth and numerous long roots *in vitro* (Yang *et al*. 2003).

Resupination, the turning of orchid buds as they open, and of newly opened flowers, is a process which positions the labellum lowermost: TIBA, morphactin and some other gravitropism-affecting chemicals inhibit resupination (Nair and Arditti 1992). The pedicel twisting may be as a result of raphide cell elongation (Dines and Bell 1994).

GENETIC TRANSFORMATION

An updated and comprehensive analysis is provided more recently in Teixeira da Silva *et al*. (2011). Many orchids are commercially important species grown for cut flowers and potted plants, and there is considerable interest in the production and improvement of these commercially valuable plants. However due to their long juvenile periods and reproductive cycles, genetic improvement using traditional sexual hybridization is restricted (Yu and Goh 2000a), as seen with *Cymbidium* in which, despite over 40 species being existent, only 5 have played a major role in producing standard-sized hybrids (Rogerson 1991a) and in the production of white *Paphiopedilums* (Rogerson 1991b). Following *in vitro* seed germination followed decades of traditional breeding that have been used in the production and improvement of the diverse array of commercially available orchids. There still exists a huge reservoir of traits, such as disease and pest resistance, stress tolerance, flower colour, scent, size and form modification, and growth habit that may be amenable for further genetic enhancement of orchids using modern breeding methods based on gene transfer technology. One of the major concerns is inducing fungal and viral resistance, the latter being primarily caused by *Cymbidium* mosaic potexvirus (CymMV) and *Odontoglossum* ringspot tobamovirus (ORTV) (Zettler *et al.* 1990; Hu *et al.* 1994), detected by reverse transcriptase-polymerase chain reaction (Eun *et al.* 2000), and eliminated in *Oncidium* through detection at the PLB level by tissue-print hybridization (Chia and He 1999). The elimination of odontoglossum ringspot virus from *Cymbidium in vitro* cultures was achieved through the culture of apical meristems in conjunction with the application of VIRAZOLE® (Toussaint *et al.* 1993).

A number of investigations explored the feasibility of incorporating selectable markers or reporter genes into orchids, including GUS (Griesbach and Hammond 1993), the non-invasive marker *luc*iferase (Chia *et al.* 1994), *bar* (gene from *Streptomyces hygroscopicus*, conferring resistance to a broad-spectrum herbicide phosphinothricin (PPT); Thompson *et al.* 1987; Gordon-Kamm *et al.* 1990) and NPTII (kanamycin resistance; Kuehnle and Sugii 1992). In the case where *bar* is used, bialaphos, an alanine derivative of PPT, is used to select transformed tissues (Gordon-Kamm *et al.* 1990). In these studies immature embryos, protocorms and PLBs served as target tissues. In addition to these antibiotic (Kuehnle and Sugii 1992; Anzai *et al.* 1996; Belarmino and Mii 2000; Yu *et al.* 2001), herbicide (Knapp *et al.* 2000) and *luc* (Chia *et al.* 1994) genes, a sweet pepper ferredoxin-like gene, *pflp*, was introduced (by *Agrobacterium* and bombardment) into *Oncidium* (You *et al.* 2003), serving as a marker gene, and conferring resistance to the soft-rot disease caused by *Erwinia carotovora*.

Orchids are naturally resistant to many of the aminoglycosidic compounds commonly used for selection of genetically transformed plant cells such as kanamycin and hygromycin, presenting further difficulty in developing selection strategies for the recovery of transformed tissues (Hauptmann *et al.* 1988), and since high levels of kanamycin are required for effective selection, chimeric plants are often obtained (Chia *et al.* 1995). The use of hygromycin results in successful discrimination of transformed and untransformed tissue (Belarmino and Mii 2000; Yu *et al.* 1999; Men *et al.* 2003). It was shown that 100-200 mg l^{-1} kanamycin is effective for stringent selection of transformed *Dendrobium* tissues, but death of non-transformed tissues requires a lengthy (3.5 months or more) selection, depending on the cultivar (Nan and Kuehnle 1995). In this sense the use of bar is more effective since bialaphos at ~ 1 mg l^{-1} effectively inhibits growth of orchid PLBs (or death at 3 mg l^{-1}) within 4 weeks of application (Knapp *et al.* 2000).

Despite transgenic orchid plants having been obtained from *Dendrobium* (Kuehnle and Sugii 1992), *Cymbidium* (Yang *et al.* 1999), *Phalaenopsis* (Anzai *et al.* 1996; Belarmino and Mii 2000), *Vanda* (Chia *et al.* 1990) and *Cattleya*, *Brassia* and *Doritaenopsis* (Knapp *et al.* 2000), transformation efficiency was relatively low in all. Actively dividing cells are shown to be more prone to transformation, hence the preference for callus cultures (Yang *et al.* 1999). Moreover early selection (~2 weeks after bombardment) resulted in heavy necrosis, whereas selection 2-3 months after bombardment increased transformation efficiency (Yu *et al.* 1999). Similarly Yang *et al.* (1999) incubated PLBs on selection-free medium for 30 days following bombardment. In contrast, Men *et al.* (2003) stated that later selection resulted in lower transformation efficiencies; in particular selection delayed up to 1 month resulted in no transformants. Embryo electrophoresis, the direct transfer of DNA into target tissues from DNA-embedded agarose, has also been used to transform *Calanthe*, whose pollen and protocorms were used as the target tissues (Griesbach and Hammond 1993; Griesbach 1994): all other studies used callus or PLBs. Tee *et al.* (2003) believe that the problem associated with using orchid embryos or protocorms is resulting chimerism, so that embryogenic callus is desirable in a reliable transformation system as plant regeneration proceeds directly without intervening callus or a *de novo* embryogenesis phase during wich non-transformed cells might be more effectively eliminated (Kuehnle and Sugii 1992).

Agrobacterium-mediated transformation

Transformation of monocotyledonous plants is traditionally thought to be difficult, but the success of *Dendrobium* (Nan *et al.* 1997; Yu *et al.* 2001) and *Oncidium* (Liau *et al.* 2003a, 2003b) transformation was attributed to the acidic nature of co-cultivation medium that might activate *Agrobacterium vir*(ulence) genes and to the presence of the *vir* gene inducer, coniferyl alcohol. Coniferyl alcohol is present at a higher level in PLBs than in other tissues (11-fold more than in leaves), and its production or stability in PLBs is enhanced by light (Nan *et al.* 1997), suggesting that PLBs are ideal starting explants for *Agrobacterium*-mediated transformation. In both genera the use of acetosyringone greatly enhanced infectibility as did a long co-culture period. Yu *et al.* (2001) transformed *Dendrobium* 'Madame Thong-In' by inoculating thin section explants from PLBs with *Agrobacterium tumefaciens*. In these transgenic plants, selected for on a 200 mg l^{-1} kanamycin medium, expressing multiple copies of the *DOH1* (first orchid homeobox gene) antisense gene, showed the formation of fasciated shoot apical meristems (i.e. 2 shoots on the same meristem) and early flowering (Yu and Goh 2000a; Yu *et al.* 2001). Transgenes (*DOH1* antisense gene), present in one, two or sometimes multiple copies, had the same effect on the degradation of the *DOH1* mRNA, indicating that the altered phenotype of *35S::DOH1as* transgenic plants was coupled with, and possibly caused by, the reduced level of the *DOH1* mRNA, suggesting a role for the *DOH1* gene in the maintenance of basic plant architecture in orchid (Yu *et al.* 2000). In *Oncidium* transformation experiments, selection was early, 3 days after inoculation, and was done on 100 mg l^{-1} timentin, while selection of *Phalaenopsis* transgenic plants occurred at the same concentration of kanamycin after 30 days culture, but protocols were genotype-dependent (Hsieh *et al.* 1997).

The application of 200 μM acetosyringone (AS) and a 2-step cocultivation method were required for successful *Phalaenopsis* transformation (Belarmino and Mii 2000), provided extremely high concentrations (200 mg l^{-1}) of hygromycin or geneticin were applied. In this study, the addition of *Agrobacterium*-removing agent, cefotaxime, at 500 mg l^{-1} or higher resulted in decreased cell suspension growth and inhibited the formation of PLBs.

Resistance against soft rot disease, caused by *Erwinia carotovora*, was engineered in *Oncidium* Sherry Baby OM8 (Liau *et al.* 2003a, 2003b).

Biolistic-mediated transformation

The first transgenic orchid plant was *Dendrobium*, generated by biolistic bombardment (**Table 4**), and transgene insertion was confirmed by kanamycin selection and PCR analysis (Kuehnle and Sugii 1992). Chia *et al.* (1994) also transformed *Dendrobium* through biolistic bombardment using tungsten particles and a non-invasive selection system, the firefly luciferase gene, although the use of an invasive (*gus*) system is most common (e.g. Steinhart *et al.* 1997). Transformation of orchids by biolistics has been reported in *Dendrobium phalaenopsis* and *D. nobile*: despite transgene expression of the transgenes in both calli and PLBs, no

Table 4 Orchid transformation studies (≤2004) For a different and complete review, see Teixeira da Silva *et al.* (2011).

Species and cultivar(s)	Transformation	Target tissue	Transgene(s)	Promoter	TrE%*
Brassia rex Sakata x *B. verucosa*	Biolistic	PLB	*Bar*	CaMV35S	n.s.
Calanthe sedenii Cornelius Vanderbilt	Electrophoresis	Protocorm	*GusA*	CaMV35S	n.s.
Cattleya	Biolistic				
Cattleya Georgiana x Self	Biolistic	Protocorm	*Bar*	CaMV35S	n.s.
Cymbidium	Biolistic	PLB	*GUS-int,nptII*, tMAR	CaMV35S	<87%
Dendrobium phalaenopsis Banyan Pink	Biolistic	C, PLB	*GUS-int,hptII*	CaMV35S	<14%
Dendrobium nobile	Biolistic	C, PLB	*GUS-int,hptII*	CaMV35S	<4%
	Agrobacterium	PLB	*GUS-int,hptII*	CaMV35S	18%
Dendrobium x Jaquelyn Thomas	Biolistic	Protocorm	*nptII,PRV-CP*	CaMV35S	4.6%
	Biolistic	PLB/P/S	*GUS,nptII*	CaMV35S, *neo*	11.7%
Dendrobium Madame Thong-In	*Agrobacterium*	PLB TCS	*nptII,Doh1as*	CaMV35S	21%
Dendrobium MiHua	Biolistic	Protocorm	*GUS-int,bar,hptII*	CaMV35S	5-10%
Dendrobium Sonia	Biolistic	PLB	*GUS,DSCKX1*	DSCKX1	n.s.
Dendrobium Sonia 17	Biolistic	C	*sgfp*	CaMV35S/HBT/Ubi1	<1000 GFP cells
Dendrobium White Angel	Biolistic	PLB	*Luc,nptII*	CaMV35S	n.s.
Doritaenopsis ***2**	Biolistic	PLB	*Bar*	CaMV35S	n.s.
Oncidium Sherry Baby OM8	Both	PLB	*GUS,hph,pflp*	CaMV35S	n.s.
	Agrobacterium	PLB	*GUS-int,hptII*	CaMV35S	2.8-10.8%
Phalaenopsis x *Doritaenopsis*	*Agrobacterium*	Cell clump	*GUS-int,nptII, hptII*	CaMV35S	<0.05%
Phalaenopsis ***1**	Biolistic				
	PTP				
	Agrobacterium	PLB	*GUS-int*	CaMV35S	50-80
Phalaenopsis Taisuco 339	Both; EP; SAAT	PLB	*GUS-int,GFP, hptII*	CaMV35S	n.s.
Phalaenopsis T0,T5,T10,Hikaru	*Agrobacterium*	PLB	*GUS-int,nptII, hptII*	CaMV35S	1.5-15%
Phalaenopsis Danse x	Biolistic	PLB segment	*GUS,bar,nptII*	CaMV35S	<98 GFP
Doritaenopsis Happy Valentine			*EG*		
P. Richard Schaeffer Santa Cruz					
Vanda	Biolistic				

Species and cultivar(s)	LtTEX	LsTEX	PCR	Southern	Others	Silencing	Reference
Brassia rex Sakata x *B. verucosa*	Callus	11 plants	Yes	Yes	Northern	n.s.	Knapp *et al.* 2000
Calanthe sedenii Cornelius Vanderbilt	Seedling	>55%	Yes	No	None	n.s.	Griesbach 1994
Cattleya							Steinhart *et al.* 1997
Cattleya Georgiana x Self	Callus	3 plants	Yes	Yes	Northern	n.s.	Knapp *et al.* 2000
Cymbidium	100 GFP	~3 GFP/PLB	Yes	Yes	None	n.s.	Yang *et al.* 1999
Dendrobium phalaenopsis Banyan Pink	Callus	C/R/S/PLB	No	Yes	Northern	Yes	Men *et al.* 2003a
Dendrobium nobile	Callus	Plant	No	Yes	Northern	No	Men *et al.* 2003a
	PLB	Plant	No	Yes	GFPF	No	Men *et al.* 2003b
Dendrobium x Jaquelyn Thomas	Protocorm	Plantlet	Yes	Yes	None	n.s.	Kuehnle and Sugii 1992
	PLB/P/S	Plantlet	Yes	Yes	ELISA	n.s.	Nan and Kuehnle 1995
Dendrobium Madame Thong-In	Callus	154 plants	Yes	Yes	Northern	n.s.	Yu and Goh 2000 Yu *et al.* 2001
Dendrobium MiHua	PLB	Plant	No	Yes	GFPF	n.s.	Yu *et al.* 1999; Yang *et al.* 2003
Dendrobium Sonia	All organs	6-15 plants	No	Yes	GFPF	n.s.	Yang *et al.* 2002 2003
Dendrobium Sonia 17	Callus	None	No	No	GFPF	n.s.	Tee *et al.* 2003; Tee and Maziah 2004
Dendrobium White Angel	215 LFP	PLB	No	Yes	Northern	n.s.	Chia *et al.* 1994
Doritaenopsis ***2**	Callus	18 plants	Yes	Yes	Northern	n.s.	Knapp *et al.* 2000
Oncidium Sherry Baby OM8	Plant	17 lines	No	Yes	North/western	n.s.	You *et al.* 2003; Liau *et al.* 2003b
	PLB	Plant	No	Yes	Northern	n.s.	Liau *et al.* 2003a
Phalaenopsis x *Doritaenopsis*	Callus	34 plants	Yes	Yes	GFPF	n.s.	Belarmino and Mii 2000
Phalaenopsis ***1**							Hsieh *et al.* 1995
							Hsieh and Huang 1995
	PLB	Not tested	No	No	GFPF/SEM	n.s.	Hsieh *et al.* 1997
Phalaenopsis Taisuco 339	PLB	Not tested	No	No	GFPF	n.s.	Chan *et al.* 2003
Phalaenopsis T0,T5,T10,Hikaru	>27%	Plant	Yes	Yes	GFPF	n.s.	Chai *et al.* 2002
Phalaenopsis Danse x	~15%	Plant	Yes	Yes	Western	None	Anzai *et al.* 1996
Doritaenopsis Happy Valentine							Anzai and Tanaka 2001
P. Richard Schaeffer Santa Cruz							
Vanda							Chia *et al.* 1990

***1** = *Phalaenopsis* Brother Mirage ‘A79-69’, P. True Lady ‘A76-13’, P. Asian Elegance ‘B79-11’, P. Taisuko Kaaladian ‘F80-13’; ***2** = *Doritaenopsis* Su’s Red Lip ‘Ching Hua II’ FCC/AOS x *D.* Amour Rojo ‘Bonnie Vazquez’.TrE = transformation efficiency: № positive shoots or explants/ № explants x 100. EP = electropoation, GFP = green fluorescent protein, GUS = β-glucuronidase, *hph* = hygromycin phosphotransferase, *pflp* = sweet pepper ferredoxin-like protein, PTP = pollen-tube pathway method (Hsieh and Huang, 1995), SAAT = sonication-assisted *Agrobacterium* transformation; tMAR = tobacco matrix-attachment region; PRV-CP = papaya ringspot virus coat protein; TCS = thin cross section; TEX = transgene expression; LtTEX = localization of transient TEX, LsTEX = localization of stable TEX; EG = β-1,3-endoglucanase from soybean (Takeuchi *et al.* 1990); C = callus, P = protocorm, PLB = protocorm-like body, R = root, S = shoot; Δ=*A. rhizogenes*; G(L)FP = GUS (Luciferase) focal point; BSA = blue staining area; GFPF-GFP fluorescence; n.s. = not specified.

transgenic plants could be regenerated (Men *et al.* 2003), although antibiotic concentration and post-bombardment selection date (2 days following bombardment) affected transformation efficiency, as did pre-culture osmoticum treatment of PLBs or calli on a 0.4 M mannitol 1/2 strength MS medium. Southern analysis of both species indicated that *D. phalaenopsis* ‘Banyan Pink’ transgenic lines had low copy numbers of the integrated genes, while *D. nobile* transgenic lines had multiple transgene copy inserts. The use of different *Dendrobium* callus types (A, B, and C) resulted in different transgene expression (Tee *et al.* 2003). In this study GFP could be detected only 3 h post bombardment, with the highest expression occurring at 2 days post-bombardment. The CaMV-35S promoter, the most commonly used in orchid transformation studies (**Table 4**), was shown to express the *sgfp* gene better than the Ubi1 and HBT promoters. Transgenic *Phalaenopsis* plants were successfully produced following particle bombardment (Anzai *et al*. 1996; Anzai and Tanaka 2001) with plasmids containing the *gus*, *bar* and *EG* genes, the *bar* gene containing PAT from *Streptomyces hygroscopicus* serving as a useful selective marker for stable transformation of various monocots (Toki *et al*. 1992) and dicots (Saito *et al*. 1992) while the *EG* (β-1,3-endoglucanase) gene from soybean (Takeuchi *et al*. 1990) confers resistance to fungal disease; stable expression of the transgenes could be detected in the T_1 and T_2 progeny. The use of the *bar* gene as a selective marker was successful in generating *Brassia*, *Cattleya*, and *Doritaenopsis* transformants (Knapp *et al*. 2000).

The bombardment of *Cymbidium* PLBs derived from liquid culture showed higher transformation efficiencies than those derived from solid medium (Yang *et al*. 1999). Non-uniform incorporation of transgenes has been observed in the biolistic transformation of orchids (Kuehnle and Sugii 1992; Yang *et al*. 1999; Men *et al*. 2003). The choice of protocorms as the target tissue resulted in successful transformation of *Phalaenopsis* (Anzai *et al*. 1996), *Dendrobium* (Kuehnle and Sugii 1992) and *Vanda* (Chia *et al*. 1990).

Biolistics was used to transform *Dendrobium* Sonia PLBs with an orchid cytokinin oxidase *DSCKX1* promoter (Yang *et al*. 2002), which is regulated by cytokinins, and subsequently regulates the levels of active forms and their distribution in plant tissues.

In separate experiments, bombardment of tobacco tissues with the *Cymbidium* mosaic virus (CyMV) coat protein gene conferred protection against the virus (Chia *et al*. 1992), which is easily transmitted in *Dendrobium* seed propagation (Yuen *et al*. 1979).

CRYOPRESERVATION AND GERMPLASM STORAGE

Cryopreservation is an important method (recognized by the FAO, IPGRI, IBPGR and CGIAR) for the maintenance of important germplasm over an extended period of time. Conservation strategies of orchids whose natural habitats are endangered are enhanced when seed (considered to be "orthodox") banking as an *ex situ* preservation strategy is adopted, and their longevity is enhanced by both reducing storage moisture content from around 20% to 5% and by lowering storage temperature from 62°C (Pritchard *et al*. 1999). Moreover mature seed germination problems such as dormancy induced through the accumulation of inhibitory substances, or incapacity to germinate despite radical treatments such as sonication, may be overcome by the use of immature seeds, useful cryopreservable units. Seeds, when preserved dry, can retain viability for up to 5-20 years at refrigeration temperatures (Shoushtari *et al*. 1994), 6 years at -20 or -196°C (Pritchard and Seaton 1993), and ten years at -24 to -40°C (Thornhill and Koopowitz 1992; Koopowitz and Thornhill 1994), but storage conservation should be supported by appropriate seed viability tests such as triphenyl tetrazolium chloride (TTP; Van Waes and Debergh 1986) and density separation (Jordão *et al*. 1988). Seeds of tropical terrestrial (*Calanthe*), epiphytic (*Angraecum*, *Trichopilia*, *Miltonia*, *Encyclia*, *Eulophyella*, *Epigenium*) and hybrid (*Bratonia* = *Miltonia flavescens* × *Brassia longissima*) orchids was achieved by freezing them in plastic ampoules by rapid immersion in liquid nitrogen, stored for a month in a cryobank, thawed within 40-60 s in a 40°C water bath, and finally sowed on modified Knudson's medium withh 1 mg l^{-1} kinetin and 0.1 mg l^{-1} NAA (Nikishina *et al*. 2001; Popova *et al*. 2003). *In vitro* storage in orchid plant parts is limited, and has only been reported for *Spathoglottis* protocorms (Singh 1991), *Darwinara*, and *Brassocattleya* PLBs (Kishi and Takagi 1997a), and *Vanilla* shoots (Agrawal *et al*. 1992). It was shown that storage of orchid seeds at 4°C in $CaCl_2$ as a dessicant are suitable long term (10-20 years) storage conditions (Shoushtari *et al*. 1994). Direct cryopreservation of *Dendrobium candidum* PLBs was possible following air-drying, the success of which was attributed to an accumulation of soluble sugars, heat-stable proteins and dehydrins (Bian *et al*. 2002). *Doritaenopsis* suspension cultures could be cryopreserved using vitrification (Tsukazaki *et al*. 2000) as could *Bletilla striata* immature seeds (Hirano *et al*. 2005).

Synthetic seeds or 'synseeds' (reviewed by Sharma *et al*. 2013) have been formed by the encapsulation of *Cattleya leopoldii*, *Dendrobium* Sonia, *D. wardianum*, *Oncidium* Gower Ramsay, *Phaius tankervilliae*, *Phalaenopsis* Santa Cruz and *Geodorum densiflorum* PLBs (Redenbaugh *et al*. 1987; Sharma *et al*. 1992; Tanaka *et al*. 1993; Malemnganba *et al*. 1996; Datta *et al*. 1999; Saiprasad and Polisetty 2003), *Ipsea malabarica in vitro*-formed bulbs (Martin 2003) or *Agrostophyllum myrianthum*, *Cymbidium longifolium*, *Coelogyne odoratissima*, *Renanthera imschootiana*, and *Spathoglottis plicata* protocorms (Singh 1991, 1992; Chetia *et al*. 1998; Kamalakannan *et al*. 1999) in an alginate (with or without nutrient enrichment) matrix. Chetia *et al*. (1998) further claim that encapsulation stimulates a higher germination than controls on a simple Nitsch and agar medium. Vij *et al*. (2001) claimed that 20 species spanning 16 genera were used to produce synseeds. In the case of *Geodorum densiflorum*, a food preservative (sodium bicarbonate) and fungicide were incorporated into artificial seeds, allowing for direct transfer to non-sterile soil conditions (Datta *et al*. 1999). Similarly *Cymbidium* PLB synseeds embedded in a fungicide, and cocooned in chitosan resulted in a 35% germination rate when sown directly on non-sterilized substrate (Nhut *et al*. 2006). When orchid seeds were placed in a number of organic solvents for long-term preservation, petroleum ether was found to give the best results after 1 year, while all other solvents reduced seed viability (Singh 1988). Martin and Pradeep (2003) suggested the use of jam bottles (rather than test tubes), half-strength MS with 3% sugar and 1.5 mg l^{-1} kinetin, half-strength MS medium (PGR-free) with 3% sugar, or half-strength MS (PGR-free, sugarless, i.e. photoautotrophic) for the long-term storage (14, 20 or 27 months for the 3 media types, respectively) of *Ipsea malabarica*. Protocorms 3-4 mm in size were shown to be suitable for optimal conversion frequency of encapsulated PLBs of *Cymbidium giganteum*, smaller PLBs not being able to withstand encapsulation or requiring a long time to emerge out of the capsule (Corrie and Tandon 1993). Ishikawa *et al*. (1997) germinated seeds of Japanese terrestrial orchid, *Bletilla striata* on solidified new Dogashima (ND) medium (Tokuhara and Mii 1993) for 10 days; embryos were then precultured on ND medium supplemented with 0.3 M sucrose for 3 days at 25°C in continuous darkness, then overlaid with a 2 M glycerol and 0.4 M sucrose solution ofr 15 min at 25°C and finaly dehydrated with highly concentrated vitrification solution (PVS2) for 3 h at 0°C prior to immersion into liquid nitrogen for 30 min. Following rapid warming, embryos were washed with liquid ND medium supplemented with 1.2 M sucrose for 20 min and then plated onto ND medium, resulting in 60% plant regeneration.

About 62% of seeds from selfing of Thai *Doritis pulcherrima* could germinate following successful cryopreservation by dehydration in 2 ml cryotubes filled with highly concentrated vitrification solution (PVS2) at 25°C for 50 min, and rapid plunging into liquid nitrogen (Thammasiri 2000). *Dactylorhiza fuchsii* and *Anacamptis morio* seeds were encapsulated in alginate beads with hyphae of the basidomycete fungus *Ceratobasidium cornigerum*, and did not lose viability even after storage at -196°C for 30 days (Wood *et al*. 2000). *Eulophia gonychila*, *Dendrobium anosmum*, *Dactylorhiza fuchsii*, *D. majalis*, and *Paphiopedillum rothschildianum* could survive 12 months storage at -196°C, i.e. cryopreserved (Pritchard *et al*. 1999).

High germination frequencies of *Spathoglottis plicata* (Singh 1991), *Dendrobium wardianum* (Sharma *et al*. 1992), *Phaius tankervillae* (Malemnganba *et al*. 1996), and *Cym-*

bidium giganteum, *Dendrobium* Sonia, and *Oncidium* Gower Ramsay (Saiprasad and Polisetty, 2003) encapsulated PLBs, or *Agrostophyllum myrianthum*, *Cymbidium longifolium*, and *Renanthera imschootiana* protocorms (Chetia *et al*. 1998) occurred when they were stored at 4°C. Kishi and Takagi (1997a, 1997b) showed that *Darwinara* and *Brassocattleya* PLBs could be stored at room temperature, following gradual dessication on a laminar flow bench, without a loss of viability. Viability was 100% in *Dendrobium densiflorum* beads when used immediately after conversion, but only 30% viability could be obtained after 30 days storage at 25°C as compared to 105 days storage at 4°C (Vij *et al*. 2001). In this study a mild dehydration of the encapsulatable units (PLBs) gave them resistance against the encapsulation and conversion-related stresses. Similar viability periods were obtained for *Geodorum densiflorum* beads (Datta *et al*. 1999). Nayak *et al*. (1998) reported the possibility of storing *Spathoglottis plicata* beads for 45 days at 4°C without markedly affecting their conversion frequency. A requirement of embryo respiration of hydrated capsules might explain the loss of viability and poor storage at room temperature (Redenbaugh 1990). *Paphiopedillum insigne* leaves are chilling-resistant as a result of the plasmalemma and cell wall acting as a barrier against the propagation of extracellular ice, especially at -2°C (Yamada *et al*. 2002).

Synthetic seeds sown *ex vitro* directly in the greenhouse or on non-sterile substrata are highly susceptible to bacterial, fungal, and other infections, overcome by the inclusion of antimicrobials such as Bavistin or streptomycin or even PGRs in the alginate bead gel matrix (Vij *et al*. 1992, 2001).

The handling of beads, which are usually sticky, can be improved by the application of (sterilized) talc powder, which does not affect viability (Vij *et al*. 2001).

Callus tissues of *Mokara* and *Dendrobium* were successfully stored under low temperature (Sivasubramaniam *et al*. 1987; Lee and Lam-Chan 1993) and with a mineral oil medium overlay in the former after 6 months culture (Lee and Lam-Chan 1993).

POSTHARVEST BIOTECHNOLOGY

Keeping quality and extending vase life of cut flowers is the major concern of commercial orchid exporters (Teixeira da Silva 2004). Among the commonly cultivated varieties of the Orchidaceae, vase-life can vary from a number of days to more than 2 months while colour changes are caused by degradation of flavonoids, carotenoids, and chlorophylls (Thammasiri *et al*. 1987); in contrast chlorophyll production in *Phalaenopsis* flowers and fruits is enhanced following pollination (Tran *et al*. 1995). Ethylene is the major environmental pollutant affecting floral senescence and cut flower longevity, and is often affected by levels of the precursor ACC (Nair and Fong 1987), produced in the rostellum and stigma (Chadwick *et al*. 1986), onset by pollination (Hew *et al*. 1989) in which there is strict interorgan regulation (O'Neill *et al*. 1993) caused by short-chain saturated fatty acids (Halevy *et al*. 1996), and rapid translocation from production sites to the all flower parts where it is converted into ethylene (Woltering 1990a, 1990b; Woltering *et al*. 1995). The application of aminoethoxyvinylglycine (AVG) under low relative humidity conditions inhibited emasculation-associated phenomena in *Cymbidium* and *Phalaenopsis* flowers, such as ethylene production (Woltering and Harren 1989). Emasculation, together with exogenous ethylene treatment, incduced red colouration of *Cymbidium* lips, resulting from an increase in anthocyanin (at least 6) accumulation preceded by a pronounced increase in phenylalanine ammonia-lyase (Woltering and Somhorst 1990). In *Aranda* the onset of ethylene production induced a cyanide-resistant pathway in fully opened flowers (Yip and Hew 1989). A laser-driven photoacoustic detection system was developed to detect ethylene production in *Cymbidium* flowers (Woltering *et al*. 1988). The time to visible senescence (wilting) in open florets of a diploid line of *Dendrobium* 'Caesar' was shorter than in a tetraploid line, which exhibits a lower respiration rate (Ketsa *et al*. 2001).

Most orchid cut flower holding solutions contain 8-hydroxyquinoline sulfate (HQS; antimicrobial agent), $AgNO_3$ (ethylene inhibitor) and a sugar (sucrose) (Ketsa *et al*. 1995), which when applied in combination, can increase the vase life by more than 30% in *Oncidium* (Hew 1987). Whereas the application of BA caused an increase in the shelf-life of other cut flowers, it shortened that of *Arundina bambusifolia* (Paull and Chantrachit 2001). The application of 1-methylcyclopropane (MCP) could extend the vaselife of *Cymbidium* orchids by circa. 30% (Heyes and Johnston 1998). *Cattleya*, *Cymbidium*, *Dendrobium*, and *Oncidium* showed, in this order, an increase in sensitivity to γ irradiation, used in the elimination of pests and diseases during quarantine (Kikuchi 2000). The application of aminooxyacetic acid (AOA) or 2,5-norbomadiene (NBD), ethylene inhibitors, delayed premature senescence of *Dendrobium* flowers following the ethylene burst 9h after pollination (Ketsa and Rugkong 2000); incidentally 72% of 61 *Dendrobium* species show high incompatibilty in interspecific pollinations, unlike other orchid genera (Johansen 1990). Pollination induces ethylene biosynthesis in *Phalaenopsis* flowers causing a faster acceleration of wilting as compared to cut flowers (Porat 1994), possibly as a result of the involvement of lipoxygenase and jasmonates (Porat *et al*. 1995b), and the initiation and propagation of ethylene biosynthesis is regulated by the coordinated expression of three distinct ACC synthase genes (Bui and O'Neill 1998). Removal of open florets, or bagging of open florets reduced *Dendrobium* flower bud opening when kept in an opening, pulsing or holding solution (Ketsa and Aree 1995).

Ethylene sensitivity is species-dependent, with *Vanda* being most sensitive, intermediate in *Phalaenopsis* (Porat *et al*. 1995a), and *Dendrobium* and *Oncidium* being insensitive (Goh *et al*. 1985; Ketsa and Thampitakorn 1995), and silver thiosulphate (STS), despite being partially inhibitory to ethylene production in *Cymbidium*, did not have any effect on cut flower longevity in *Cattleya*, as neither did AOA. STS or AOA application in *Dendrobium* reduced ACC and resulted in longer vase life of cut flowers (Nair *et al*. 1991; Rattanawisalanon *et al*. 2003), which is generally reduced by cross-pollination, in which pollinia contain ACC (Ketsa and Luangsuwalai 1996). Ethylene also results in increased anthocyanin production of cut *Cymbidium* flowers (Arditti *et al*. 1973). Ethylene, often produced in response to stress, is also produced by developing racemes of *Catasetum* and *Cycnoches* (Gregg 1984). Total sugars (sucrose, glucose, and fructose) increased significantly as *Cymbidium* buds opened, although nocturnal variations were observed in fructose, and despite a constant glucose level independent of storage temperature, flowers exhibited discoloring and sensitivity to chilling temperatures (4°C; Pritchard and Hew 1998). Okadaic acid, an inhibitor of type 1 or type 2A serine/threonine protein phosphatases, when applied to *Phalaenopsis* stigmas induced a dramatic increase in ethylene production and an accelerated senescence of the whole flower, reversed by the application of $AgNO_3$ or AVG (Wang *et al*. 2001). AVG was also shown to prolong (150-229%) the vase life of cut *Cymbidium* flowers when applied at 1 mM (Kwack *et al*. 1996). Cut *Phalaenopsis* flowers, which usually last about 2 weeks suffer a rapid acceleration of the wilting process as a result of post-pollination ethylene-induced senescence (Porat *et al*. 1994a, 1994b), which can be delayed by treatment with $AgNO_3$ or MCP (Porat *et al*. 1995a). The rostellar-stigmatic region of *Cymbidium* flowers placed on NAA-supplemented medium was found to control post-pollination phenomena (Arditti and Flick 1976). Interestingly, despite the presence of stomata in different flowering parts, they do not serve for transpiration and are non-functional (Hew *et al*. 1980). This is also probably related to air-quality within the packaging atmosphere, which also affects the level of discoloration (Vergano and Pertuit 1993).

Disinfestation

Insecticidal dips used to eliminate both nymphs and adults were shown to significantly reduce the shelf-life of *Dendrobium* cut flowers and *Arundina* foliage (Hansen *et al.* 1992). In order to efficiently eliminate eight species of insect and arthropod pests from cut orchid flowers, a gas fumigation of 13 g m^{-3} methyl bromide, 3 g m^{-3} phosphine, 5% CO_2 with 40% loading at 15°C for 4 h and 20°C for 3 h (Kawakami *et al.* 1996). Western flower thrips and melon thrips can be eliminated by 94 and 92%, respectively in *Dendrobium* blossoms with an insecticidal fog consisting of abamectin (2%), or abamectin mixed with Pyrenone (6% pyrethrins, 60% piperonyl butoxide) without showing phytotoxicity; all other methodologies either eliminated little thrips, or were phytotoxic to some extent (Hara *et al.* 1993, 1995). The continued application of chloramphenicol in boiled deionized water was found to be most effective in increasing *Dendrobium* cut flower vase life (Dai and Paull 1991).

PHYSIOLOGY AND AGRONOMY

The physiology (photosynthesis, partitioning of photoassimilates, respiration, water relations, mineral nutrition, growth and development) of *Cymbidium sinense* (Pan *et al.* 1997) and *Oncidium* (Li *et al.* 2002a) have been well studied. PLBs have been used for studies of the effects of initial pH on the growth of orchid plantlets (Piriyakanjanakul and Vajrabhaya 1980) and the optimal composition or culture media (Ichihashi and Uehara 1987). Orchid (*Grammatophyllum speciosum*) roots, being ageotropic and sometimes negatively geotropic, serve as excellent models for geotropism studies (Churchill *et al.* 1972). Roots of *Brassia*, *Epidendrum*, *Oncidium*, and *Vanda* lack statoliths (Tischler 1905), while those of many orchids are green, and perform photosynthesis.

Symbiotic relationships

All orchids have an obligate relationship with mycorrhizal symbionts, which induce increases in nuclear size and content of the host orchid (Sentilkumar and Krishnamurthy 1999). The velamen of orchid roots produce anthocyanins (Pridgeon 1987), pectin and cellulose are produced by the plasmalemma (Nieuwdorp 1972), infected *Cymbidium* protocorms had increased levels of polyphenol oxidase (Pais and Barroso 1983), uricase (Barroso *et al.* 1988), ascorbic acid oxidase, peroxidase and catalase (Blakeman *et al.* 1976), while phytoalexins are present in the roots of many species following the establishment of mycorrhizae (Stoessl and Arditti 1984), which can be established *in vitro* (Chang and Chou 2001), while vitamin production by the symbionts affects the overall success of orchid germination (Hijner and Arditti 1973). In general, and in brief, symbiotic fungal hyphae enter the embryo of imbibed seeds either through the suspensor or epidermal hairs and form coiled, branched structures, the pelotons, in parecnhyma cells of the embryo; the peloton is separated from the parenchyma cell cytoplasm by a perifungal membrane and interfacial matrix material; colonization of embryo cells induces nuclear hypertrophy, partly due to an increase in DNA synthesis, and changes in both microtubules and actin filaments; pelotons undergo degradation and during this phase pectins, β-1,3 glucans and cellulose are deposited in the interfacial matrix surrounding senescing hyphae and hyphal clumps (Peterson *et al.* 1998). The symbiotic fungus *Ceratobasidium cornigerum*, when invading the suspensor end of *Spiranthes sinensis* embryos (Uetake and Peterson 1998), form typical hyphal coils (pelotons) within parenchyma cells, undergo lysis resulting in degraded hyphal masses, which are enveloped by host-derived membrane, and accompanied by changes (changes in the array) in actin filaments with symbiotic fungal colonization, and senescence occurring concomitantly with the changes in endoplasmic reticulum (Uetake and Peterson 1997, 2000). A temperature optimum exists for the orchid/fungus relationship in mycorrhizal fungus (*Rhizoctonia*)-assisted *in vitro* germination and seedling development in *Dactylorhiza majalis*, which is density, temperature and light-dependent (Rasmussen *et al.* 1990a, 1990b; Kohara *et al.* 1996). Infection of *Erythrorchis ochobiensis* occurred independent of the use of orchid or non-orchid symbiotic fungi (Umata 1998). A sugar input is required for *Cypripedium* seed development into protocorms, usually provided by a fungal symbiont (Leroux *et al.* 1995). Scarification of the testa in $Ca(OCl)_2$, an initial incubation for several weeks at 20°C, and subsequent cold stratification for 8-12 weeks at 4-8°C resulted in >50% germination responses and finally normal protocorm and organ development in a species usually difficult to germinate *in vitro*, *Epipactis palustris*, a terrestrial orchid (Rasmussen 1992). Chilling of *Spiranthes cernua* and *Godyera pubescens* seeds, followed by inoculation with *Platanthera ciliaris* results in successful seed germination (Zettler and McInnis 1993). The use of *Ceratorhiza* and *Epulorhiza* endophyes stimulated *Orchis morio*, *Dactylorhiza praetermissa* var. *junialis*, *D. majalis*, and *D. incarnata* growth at relatively low nitrogen availability in the culture medium (Dijk and Eck 1995). Low levels of paclobutrazol stimulated early tuberization of symbiotic orchid, *Diuris laxiflora* (Hollick *et al.* 2002). The use of high nitrogen levels turns the normally symbiotic relationship between *Dactylorhiza incarnata* protocorms in shaking cultures and *Rhizoctonia* strains into a parasitic one (Beyrle *et al.* 1991). On a separate front, Yanagawa *et al.* (1995) claimed that theb incorporation of 0.01% sodium hypochlorite or hydrogen peroxide into the growth medium allowed for *in vitro* culture under non-sterile conditions without phytotoxic effects on the seeds or plants. Sucrose-free chitosan-alginate encapsulated *Spathoglottis plicata* seeds could germinate (66-84%) *in vitro* following a symbiotic relationship with *Rhizoctonia* AM9 (Tan *et al.* 1998).

Photosynthesis

Crassulacean acid metabolism (CAM), an adaptive mechanism found in xerophytic plants and epiphytic orchids including *Cattleya* (Avadhani *et al.* 1980), facilitates regrowth after periods of suboptimal conditions, such as drought, which in turn causes a reduction in titratable acidity and a decrease in nocturnal CO_2 uptake (Fu and Hew 1982). Orchid pseudobulbs are water, mineral and carbohydrate storage organs, which allows the orchid to survive in the harsh, nutrient limited epiphytic biotope (Ng and Hew 2000). Internal CO_2 gas concentration in *Cattleya* reached in excess of 2% during the day (Cockburn *et al.* 1979). *Cymbidium* (terrestrial C_3 plant) and *Dendrobium* PLBs can grow autotrophically (without exogenous sucrose) *in vitro* under high light and CO_2-enrichment (Kirdmanee *et al.* 1992; Lim *et al.* 1992). Plantlet growth was greatly enhanced when Mokara Yellow (*Arachnis hookeriana* × *Ascocenda* Madame Kenny) were placed at super-elevated (1%) CO_2 conditions (Gouk *et al.* 1999), particularly at 5% CO_2 a 170% increase in dry mass, a higher root:shoot ratio, 373% increase in leaf area, 75% increase in soluble protein, and a 12-90% and 27-90% decrease in Rubisco and PEP-carboxylase activity, respectively (Gouk *et al.* 1997). PLBs were produced from leaf bases of *Cymbidium* 'Burgundian Chateau', accumulation of endogenous free sugar reached peaks after 3-5 and 25 days in culture while starch synthesis reached a maximum 20 days after the start of culture (Arditti and Ernst 1993). A high photosynthetic photon flux (PPF), high CO_2 concentration and increased number of air exchanges (i.e. photoautotrophic culture conditions) promoted growth and increased photosynthesis in *Phalaenopsis*, *Neofinetia falcata*, *Cymbidium kanran* and *C. goeringii* (Kirdmanee *et al.* 1992; Hahn and Paek 2001). Tissues obtained from apical meristems of *Dendrobium* 'Multico White' could only use glucose, fructose, and sucrose as carbon sources; both growth and respiration rates increased with increasing sugar levels (Hew *et al*. 1988). Sucrose-phosphate synthase (SPS) is one of the key regulatory enzymes in carbon assimilation and partitioning in plants, playing a crucial role in the production of sucrose in photosynthetic cells. The high level expression of a full-length SPS cDNA encoding SPS from *Oncidium* Goldiana, *sps1*, in flowers suggestes that it might play an important role in flowering; moreover growth under higher irradiance and elevated CO_2 leads to an accumulation of the *sps1* transcript in the photosynthetic leaves, associated with the leaf photosynthetic rate (Li *et al*. 2003a, 2003b). In a separate experiment, the physiological absorption factors influencing the improvement of indoor air quality and the difference in removal efficiency of air pollutants among four orchids, *Cymbidium virescens*, *C. kanran*, *C. sinense*, and *C. rubrigemmum*, were investigated; it was shown that the latter two had a higher removal ability of SO_2 while the former two could remove O_3 well (Han *et al*. 2002). High PPF *in vitro* resulted in higher (38%) *Phalaenopsis* fresh weight, wider leaves and more roots than at low PPF (Konow and Wang 2001). Greenhouse studies on *Sophrolaeliocattleya* (CAM) and *Cymbidium* (C_3), the former with a greater potential to adapt to high photosynthetic fluxes than the latter, showed big fluctuations in activities of foliar antioxidative enzymes (superoxide dismutase-SOD, ascorbate peroxidase-APX, carnitine acylcarnitine translocase-CAT) and a decrease in foliar chlorophyll content with an increasing radiant flux (Li J *et al*. 2001). The mykotrophic orchid, *Neottia nidus-avis* is unable to catalyze photosynthesis despite having Chl *a* and xanthophyll, resulting in yellowish-brown leaves since part of the carotenoid absorption is shifted into the green spectral region (Menke and Schmidt 1976). In *Paphiopedilum*, in contrast, despite the reduced levels of stomatal conductance when irradiated with red light, this defficiency can be corrected (up to 77% more growth) when plantlets are irradiated with blue light (Zeiger *et al*. 1985).

Nutrition

Unusual and specialized nutrition is a hallmark of the orchids. One such feature are the "shootless" epiphytic orchids in which the entire shoot represnts only a few percent of the total body mass, allowing material resources to be used for a high regenerative capacity (Benzing and Ott 1981). Inflorescence growth in monopodial orchids was shown to be primarily source-limited although significant sink limitations for assimilate gain by the inflorescence exist because of a modulating effect of the vegetative apical shoot on inflorescence sink strength and the ability of source leaves to respond positively to increased sink demand (Clifford *et al*. 1995).

OTHER ADVANCES

Growth vessels

Tissue culture in sealed membrane (polypropylene) vessels increases market access for plant products due to their relatively cheap unit cost (*c*. 170X cheaper) in comparison to regular GA7 (Magenta) flasks (Lee and Lam-Chan 1995). Orchids, especially *Cattleya* (and their clones, *Sophrolaeliocattleya* and *Brassolaeliocattleya*), have been used to study liquid/membrane culture (Hew *et al*. 1990; Adelberg *et al*. 1992, 1997). Microporous polypropylene membranes have been used for the germination of *Cattleya* and *Epidendrum*, for propagation of *Cattleya*, *Cymbidium* and *Dendrobium* PLBs, and for plantlet production with *Cattleya* and *Phalaenopsis* (Hew *et al*. 1990; Adelberg *et al*. 1992, 1997). Due to the epiphytic nature of *Cattleya*, root systems can form above a moistened membrane surface without the need for a solid matrix. Differences in growth was also attributed to the different inorganic nutrient formulations, with MS (full, half-, or quarter strength) resulting in greatest fresh weight and number of plants per vessel (Adelberg *et al*. 1997). Using hydroponic culture in sealed 3-D, polypropylene vessels with microporous, semipermeable membrane films, *Cattleya* and *Brassolaeliocattleya* could be successfully grown *in vitro* and shipped (Adelberg *et al*. 1998). *Potinara* and *Cymbidium* orchids grew at accelerated rates in liquid culture systems (plantlets supported on glass beads) where media were cycled by ebb and flow, i.e. automated media supplementation system (Tisserat and Vandercook 1986; Takano *et al*. 1990). Neoflon® films were successfully used in the Culture Pack® to vegetatively micropropagate *Cymbidium* and Culture Bag® systems to proliferate PLBs (Tanaka 1991). Park *et al*. (1996) developed a cotton-wool based PLB-induction unit that involved the induction of PLBs from *Phalaenopsis* flower stalk cultures on an inverted petri dish within a wider dish.

CO_2 enrichment

Conventional culture systems are characterized by high humidity, constant temperature, low photosynthetic photon flux density, large diurnal flucturations in CO_2 concentration, presence of high concentrations of sugars, salts and PGRs, and ethylene accumulation – conditions all affecting the uptake of water, nutrients and CO_2, transpiration, dark respiration and development of the photosynthetic machinery, resulting in poor plantlet growth (Kozai 1991). Benefits of the use of carbon dioxide enrichment (CDE) in *in vitro* culture of plantlets include: a) increased photosynthesis by chlorophyllous tissue as the CO_2 concentration within the culture vessels has been observed to decrease during the photoperiod in conventional airtight containers; b) insufficient CO_2 within the vessel limits photosynthesis during the photoperiod; c) tissues develop photoautotrophically and grow better on sugar-free medium in the presence of high CO_2 and photosynthetic photon flux density (PPFD) than under heterotrophic or mixotrophic conditions (Jeong *et al*. 1995). *Dendrobium phalaenopsis* plants only grew more under photoautotrophic conditions and CO_2 enrichment when also enriched with O_2 (Doi *et al*. 1992; Mitra *et al*. 1998) while increased growth of an *in vitro*-propagated CAM orchid hybrid Mokara White was obtained with CO_2 enrichment in an optimized photoautotrophic open system (Hew *et al*. 1995), or for *Cymbidium* in a low PPFP-high CO_2 culture system, the Miracle Pack® (Kozai *et al*. 1990; Tanaka *et al*. 1999). A doubled CO_2 concentration resulted in a 25% relative increase in growth rate of Mokara Yellow or of *Oncidium goldiana* (Li CR *et al*. 2001); a daytime down-regulation of Rubisco and PEP-carboxylase as well as a simultaneous increase in leaf sucrose phosphate synthase and sucrose synthase enhanced both the photosynthetic capacity at high CO_2 concentration and reduced resource investment in excessive Rubisco activity; there was an increase in IAA, GA_1 and GA_3, isopentenyladenosine (iPA) and zeatin riboside (ZR) in expanding leaves (Li *et al*. 2002b).

Despite increasing urbanization and industrialization, the effects of ozone and SO_2 were found to be minimal on *Encyclia* and *Epidendrum* physiological processes (Nyman *et al*. 1990).

Light source

A comprehensive comparative study was conducted to determine the effect of different lighting sources for the successful growth of orchids indoors (Poole and Seeley 1977). The use of 75% red + 25% blue light-emitting diodes (LEDs) was best for the proliferation of *Cymbidium* callus, while highest PLB production from callus was obtained when 25% red and 75% blue LEDs were used (Huan and Tanaka 2004). The use of superbright red and blue LEDs were shown to enhance *Cymbidium* plantlet growth *in vitro* under CO_2 enrichment (Tanaka *et al*. 1998) and morphogenesis of *Phalaenopsis* (Tanaka *et al*. 2001) and *Cymbidium* (Nhut *et al*. 2005) PLB segments.

The acclimatization of *Dendrobium wardianum* was successful when charcoal pieces, brick bats and coconut fibres were used (Sharma and Tandon 1992), while *Dioscorea floribunda* could be acclimatized (made autotrophic and hardened) by growing them for a few days in an inorganic salt solution before transplantation to soil (Chaturvedi 1975). Greenhouse *Phalaenopsis*, when exposed to stimulatory treatments such as low temperatures, high light intensity and CO_2 enrichment (1000-3000 ppm) at 20°C, increases the sucrose content of leaves 2-3 weeks after the start of treatment, reducing the days to spiking (Kataoka *et al*. 2004).

Robotization and bioreactors

Robotization of orchid protocorm transplanting in tissue culture has been achieved (Okamoto 1996). Tisserat and Vandercook (1986) successfully devised a computerized system that allowed the mass production of *Potinara* sp. under continuous sterile conditions. There exists only a single study that uses PLBs to mass proliferate PLBs in bioreactor system, temporary and continuous immersion (air lift column and air lift-balloon) (Young *et al*. 2000). Previous studies with rudimentary aeration systems showed that exogenously supplied filtered air increased weight and growth rate of *Aranda* and *Aranthera* protocorms propagated in liquid medium (Cheng *et al*. 1978).

Space research

Studies of epiphytic orchids in space showed that they are more resistant to long-term cultivation in orbital stations than terrestrial orchids (Cherevchenko *et al*. 1996). These model experiments on clinostats in monopodial orchids (*Vanda watsonii*, *Angraecum dictichum*), sympodial species (*Epidendrum rigidum*, *Doritis pulcherrima*), orchids with short stems (*Paphiopedilum insigne*, *Stenorhynchus* spp.), and species with pseudobulbs (*Cymbidium hybridum*, *Dendrobium crumenatum*) showed that, after long periods of clinostating, the activity of endogenous PGRs in epiphytic orchids changes less than in terrestrial plants.

PHYTOCHEMISTRY

Orchids, the doyens among ornamentals, are one of the most important global cut flowers and ornamental potting plants and some genera such as *Vanilla*, *Gastrodia*, *Bletilla*, *Dendrobium*, *inter alia*, are also used as important medicinal resources (Kimura and Kimura 1991). The secondary metabolites of the Orchidaceae plants has been intensively investigated and various constituents have been reported (Kong *et al*. 2003).

Phenanthrene derivatives are constituents common among orchid plants. Considerable amounts of both hydroxylated phenanthrenes and 9,10-dihydrophenanthrenes have been found in bulbs, roots and rhizomes. Unlike the biosynthesis of phenanthrenes from stilbenes originating from cinnamic acid derivatives, the pathway to 9,10-dihydrophenanthrenes proceeds via phenylpropionic acid derivatives and includes at least the following steps (Preisig-Müller *et al*. 1995): formation of trihydroxybibenzyl from *m*-hydroxyphenylpropionyl-CoA and three molecules of malonyl-CoA, monomethylation of the bibenzyl by an *S*-adenosylmethionine-dependent *O*-methyltransferase, a methionine-regenerating system, and an oxidative step transforming the bibenzyl into a dihydrophenanthrene. In young plants the pathway leading to 9,10-dihydrophenanthrenes, formed from bibenzyls in an oxidative coupling reaction, proceeds with increased conversion rates upon induction by fungal elicitors, such as occurs in *Botrytis cinerea* when applied to sterile plants of *Phalaenopsis* sp. (Reinecke and Kindl 1993, 1994a). Other bibenzyls have been found in *Dioscorea* species (Hashimoto and Hasegawa 1974), characterized as batatasins, related to the dormancy of bulbs (Coxon *et al*. 1982), implicated as PGRs (Pryce, 1971), or in the case of bibenzyl derivatives, including the bibenzyl carboxylic acid lunularic acid, found in large quantities in liverwort "oil bodies" (Gorham 1977).

Species-by-species characterization

1. Anoectochilus

A. formosanus (Mandarin: Jin-xian-lian; Japanese: Kinsen-ran), a terrestrial orchid, is an important medicinal herb. In Taiwanese folk medicine, the whole plant, fresh or dried, is boiled in water and taken internally in the treatment of chest and abdominal pains, diabetes, nephritis, fever, hypertension, impotence, liver and spleen disorders, and pleurodynia (Shiau *et al*. 2002). The herb is applied externally for snakebite since it contains substances that affect the arachidonic acid metabolism, involviong the functioning of the cardiovascular system (Mak *et al*. 1990). The aqueous extract of *A. formosanus* was found to possess anti-viral (Chan *et al*. 1994), anti-inflammatory, and liver-protective properties (Lin *et al*. 1993). The extract of dried *A. formosanus* was found to contain 4-hydroxycinnamic acid, β-sitosterol, β-D-glucopyranoside (Takatsuki *et al*. 1992) and three butanoic acid glucosides (Du *et al*. 1998). A compound 3-(*R*)-3-β-D-glucopyranosyloxybutanolide, named Kinsenoside isolated from *A. formosanus* and *A. koshunensis*, was found to possess an anti-hyperliposis effect (Du *et al*. 2001). A patent was registered for anti-diabetic and anti-atherosclerotic properties of compounds isolated from aqueous extract of *A. formosanus* (Takeshita *et al*. 1995).

A. formosanus is a slow growing perennial herb with seedlings that mature and reproduce through seeds after 2-3 years of growth. It flowers only once a year in the winter between October and December. Indiscriminate collection of these plants, often before they have a chance to bloom, has reduced the species towards rarity. Reduced population size of *A. formosanus* may lead to reduced gene flow, inbreeding depression, and reduced fitness. Due to high cost of the herb (the current market price of the fresh and dry herb collected from its natural habitat is around US$320 and $3,200/kg, respectively) and increasing demand, other related species such as *A. koshunensis*, *Goodyera* spp., and *Zebrina pendula* are often found as adulterants in the drug market.

2. Aranda

Four isoforms of polyphenol oxidase, responsible for tissue browning, and produced in response to mycorrhizal infection, were isolated from aerial roots of *Aranda* 'Christine 130' (Lam and Ho 1990; Ho 1999).

3. Bletilla

Bletilla striata is a medicinally important plant in China (Yam and Weatherhead 1991a, 1991b). Fungal infection of the orchid rhizomes leads to an increase in bibenzyls and 9,10-dihydrophenanthrenes, casuing a simultaneous increase in bibenzyl synthase activity (Reinecke and Kindl 1994b).

4. Bulbophyllum

B. vaginatum is distributed in Peninsular Thailand and Malaysia, Sumatra, Bangka, Java, Borneo and Maluku. Phenanthrenes, dihydrophenanthrenes and bibenzyls were isolated from the orchid *B. vaginatum* (Leong *et al*. 1997, 1999) while alkaloids have only been isolated in few *Bulbophyllum* species, while *B. leopardium*, *B. gymnopus*, *B. fuscopurpureum*, *B. guttulatum*, *B. odoratissimum* and *B. triste* have mainly yielded phenanthrene derivatives. A total of 28 compounds comprising 53, 74 and 86% of the headspace volatiles were isolated from *B. weddellii*, *B. involutum* and *B. ipanemense*, respectively (da Silva *et al*. 1999) as a first step to studying orchid-pollinator relationships. Headspace coupled to GC-MS was also used to idetify the fragrance of *Anathallis* (*Pleurothallis*) *racemiflora*, which showed a diurnal variation of most compounds, especially α-pinene, in which greater concentrations were produced during the day (Damon *et al*. 2002). The floral fragrance of *Catasetum maculatum* consists of a variety of terpenes and simple aromatics, but the main constituent is *trans*-carvone oxide (Lindquist *et al*. 1985).

Several orchid species in the genus *Bulbophyllum* produce floral fragrances that attract specific species of *Bactrocera* fruit flies (Tephritidae) for pollination (Tan *et al*. 2002) or *Euglossine* bees (Gerlach and Schill 1991). These so-called "fruit fly orchids" possess a mechanical device with a movable seesaw lip to temporarily trap a fruit fly during pollination. The fly receives a pollinarium on the thorax and transfers it to another flower of the same species mediated by the synomonal fragrance, whose primary attractive components are: methyl eugenol, 2-allyl-4,5-dimethoxyphenol, and its *O*-methyl ether, euasarone, compounds which the males then transform into the female-attracting pheremones, 2-allyl-4,5-dimethoxyphenol and *trans*-coniferyl alcohol (Tan *et al*. 2002; Nishida *et al*. 2004).

5. Coelogyne

A bibenzyl, 9,10-dihydrophenanthrene derivatives and sterols have been isolated from *C. ovalis* (Sachdev and Kulshreshthra 1986).

6. Cymbidium

The spring orchid (*Cymbidium goeringii*) is one of the most popular terrestrial species indigenous to temperate Eastern Asia, cultivated as an ornamental, and the flowers sometimes used as ingredients of a soup, alcoholic drink, or tea (Shimasaki and Uemoto 1991). The mannose-specific plant lectins from *Cymbidium* hybrid are potent and selective inhibitors of human immunodeficiency virus types 1 and 2 (HIV-1,2) in MT-4, and showed a marked anti-human cytomegalovirus (CMV), respiratory syncytial virus (RSV) and influenza A virus activity in HEL, HeLA and MDCK cells, respectively *in vitro* (Balzarini *et al*. 1992). A phenanthraquinone, cymbinodin A from *C. aloifolium* was isolated (Barua *et al*. 1990). Various phytoalexins, primarily sterols, were isolated from *Cymbidium* pseudobulbs (Arditti *et al*. 1975).

7. Cypripedium

Floral fragrances were shown to be markedly different in three separate taxa of *C. calceolus*, composed mainly of fatty acid derivatives, isoprenoids, and phenyl derivatives, respectively (Bergström *et al*. 1992), making floral fragrance a useful taxonomic tool.

8. Dendrobium

Several species of *Dendrobium* are used in traditional Chinese medicine to nourish the stomach, promote the secretion of saliva, and reduce fever (Ye *et al*. 2002). The stems of *D. nobile* (in Chinese, Jin-chai-shi-hu) is used as a Yin tonic. Various compounds identified from *D. nobile* possess antitumor and antimutagenic activity, such as gigantol (Miyazawa *et al*. 1997). The pseudobulbs of *D. tokai* are used as oral contraceptives in India (Alam *et al*. 2002). Bibenzyls and their derivatives were isolated from the orchid *D. amoenum* (Majumder *et al*. 1999a), as well as bibenzyl structural analogues moscatilin (Majumder and Sen 1987) and 3,4'-dihydroxy-5-methoxybibenzyl (Crombie and Crombie 1982; Crombie and Jamieson 1982), the picrotoxin group of sesquiterpenoids, amotin and amoenin (Dahmen and Leander 1978), and the 9,10-dihydrophenanthropyran, flaccidin/amoenumin (Veerraju *et al*. 1989). Rotundatin, a 9,10-dihydrophenanthrene derivative was isolated from *D. rotundatum* (Majumder and Pal 1992).

Dendrobium nobile contains some important alkaloids such as dendramine, nobilonine. Rare anthocyanin, cyanidin 3-(6-malonylglucoside)-7,3'-di(6-sinapylglucoside) and the demalonyl derivative were identified from *Dendrobium* 'Pompadour' (Williams *et al*. 2002); in most *Dendrobium*, the primary flavonoids are quercetin and kaempferol. Pigment distribution and epidermal cell shape determine colour intensity, perception and visual texture of *Dendrobium* flowers (Mudalige *et al*. 2003). Erianin and chrysotoxine, potent anti-angiogenic agents displaying apoptotic-inducing effects in carcinoma cells (Li *et al*. 2001), were isolated from *D. chrysotoxum* (Ma *et al*. 1994; Ma and LeBlanc 1998; Gong *et al*. 2004), which is often used as an antipyretic and an analgesic in traditional Chinese medicine. Structurally similar to erianin, phenanthrene derivatives displaying potent anti-tumor activity were isolated from *D. nobile* (Lee *et al*. 1995). Certain compounds (gigantol, moscatilin, homoeriodictyol, scoparone, scopoletin) from *D. densiflorum* exhibit anti-platelet aggregation activity *in vitro* (Fan *et al*. 2001).

Herba Dendrobii (Shihu) is a traditional Chinese medicine for the stomache, promoting the production of body fluid, nourishing "yin" and eliminating "evil-heat"and is derived from the stem of five *Dendrobium* species: *D. candidum*, *D. chrysanthum*, *D. fimbriatum*, *D. loddigesii*, and *D. nobile* (Lau *et al*. 2001). rDNA ITS were used to authenticate these medicinal *Dendrobium* species, which are rather expensive and in which adulteration by the use of more common orchids such as *Pholidota cantonensis* is frequent; this is required to protect consumers since the low (1%) intra-specific variation among the species alows the 2 ITS regions to be adopted as a molecular marker for differentiating medicinal *Dendrobium* species, and to support conservation measures (Lau *et al*. 2001).

9. Epidendrum

The floral fragrance of *E. ciliare* revealed that β-pinene and Δ-3-carene are the major component of young flowers, while γ-terpinene levels are high in older flowers (Moya and Ackerman 1993).

10. Gastrodia

The corm of *Gastrodia* elata has been widely used as a tranquilizer and anodyne with no side effects in Chinese medicinal science for about 2,000 years. An anti-fungal protein GAFP-1 (*Gastrodia* anti-fungal protein, also called gastrodianin) was purified from *G. elata*, a parasitic plant on the fungus *Armillaria mellea* (Xu *et al*. 1998; Liu *et al*. 2002). GAFP-1, induced in the nutritive corm and accumulated in the terminal corm, inhibits the hyphal growth of some phytopathogenic fungi such as *Valsa ambiens*, *Rhizoctonia solani*, *Gibberella zeae*, *Ganoderma lucidum* and *Botrytis cinerea in vitro*; the amino acid sequence of the N-terminal of GAFP-1 shared high homology with those of other lectins from orchids such as *Cymbidium hybridum*, *Epipactis helleborine Laelia autumnalis*, and *Listeria ovata* (Van Damme *et al*. 1987, 1994; Kaku *et al*. 1990; Saito *et al*. 1993; Zenteno *et al*. 1995). Lectins, proteins that specifically and reversibly bind carbohydrates and agglutinate cells, are useful tools for the isolation and characterization of well-defined glycan structures and cellular subsets (Osawa and Tsuji 1987).

11. Maxillaria

Maxillaria densa is an epiphytic orchid widely distributed in Mexico and Guatemala from which several phenanthrene derivatives (Estrada *et al*. 1999; Valencia-Islas *et al*. 2002) were isolated; despite being phytotoxic against *Amaranthus* and *Lemna* (weeds), they also exhibit moderate toxicity in all mammalian cells tested, thus they cannot be used as herbicides. Similar compounds, namely gymnopusin, were isolated from *Bulbophyllum gymnopus* (Majumder and Banerjee 1989) and erianthridin from *Eria* spp. (Majumder and Joardar 1985).

12. *Ophrys*

Flowers of the genus *Ophrys* resemble female insects, and thereby sexually deceive, attract and are pollinated by male insects. Ayasse *et al*. (2000, 2001) demonstrated through GC-MS and GC-EAG that hydrocarbons (C21-C29 alkanes and alkenes), aldehydes and esters were both the sex pheremone of the female bee and the sex attractant of the flower (Schiestl *et al*. 1999) while alkanes and alkenes together constitute the bees' sex pheremone as well as the pseudo-copulation-behaviour releasing orchid odour bouquet (Schiestl *et al*. 2000). In specific (ω-1)-hydroxy and (ω-1)-oxo acids, especially 9-hydroxydecanoic acid, are the major components of the female sex pheremone in the scoliid wasp *Campsoscolia ciliata* and honeybees, stimulating male copulatory behaviour (Ayasse *et al*. 2003). In other *Ophrys* species, the mimetic attractants were shown to be the main constituents of the floral fragrance, namely 2-heptanol, 2-nonanol, 2-hetanone, 2-nonanone, α-nonanone and α-pinene (Borg-Karlson and Groth 1986).

13. *Phalaenopsis*

Secondary metabolites of *P. equestris*, a popular potting orchid in Japan, were investigated, and two phenanthropyran derivatives were found (Manako *et al*. 2001). Shoot tips of *Phalaenopsis* are occasionally inhibited in *in vitro* culture, and this has been attributed to the phyto-inhibitory phalaenopsine T (Fujieda *et al*. 1988).

14. *Rhynchostylis*

The alcoholic extract of *R. retusa* showed a strong antibacterial effect against *Bacillus subtilis* and *Escherichia coli*, and a weaker effect with *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Salmonella typhi* (Ghanaksh and Kaushik 1999).

15. *Vanilla*

The only orchid not grown for its ornamental value, *Vanilla* is the source of the spice and flavor compound vanillin (4-hydroxy-3-methoxybenzaldehyde; Lawler 1984) and precursors such as 4-hydroxybenzaldehyde, which are obtained from the beans. Currently the major producer is Madagascar with an estimated world consumption of over 2000 tons, but the plant is attacked by a systemic fungus (*Fusarium batatatis* var. *vanillae* Tucker) that is transmitted by cuttings (Philip and Nainar 1986). Over 250 different compounds have been isolated from vanilla beans, including 4-hydroxybenzoic acid and 4-hydroxy-3-methoxybenzoic acid (vanillic acid) (Guarino and Brown 1985; Dignum *et al*. 2001). Vanilla beans are harvested up to 8 months post-pollination; at this stage the green beans are flavourless but contain large quantities of glucosides of the various flavour compounds. The characteristic flavour develops on "curing" of the beans, a process that can last for as much as 6 months and which is associated with increases in hydrolytic enzymes such as glycosidases, esterases, proteases and lipases, and oxidative enzymes such as polyphenol oxidases and peroxidases (Dignum *et al*. 2001). During curing the various glycosides are hydrolyzed and also undergo oxidation. There is extensive literature documenting the formation in plants of benzoic acids and aldehydes from hydroxycinnamic acids derived from phenylalanine via the phenylpropanoid pathway of secondary metabolism (e.g. Verberne *et al*. 1999). However considering the wide usage of vanilla flavour, and the importance of benzoic acids in the biosynthesis of molecules as diverse as the plant defense signal molecule salicylic acid, the napthoquinone pigment shikonin, cocaine, xanthones with anti-HIV activity, and the anticancer drug taxol (Podstolski *et al*. 2002, and references therein), there is still considerable uncertainty as to the nature of the biochemical reactions leading to shortening of the side chain of hydroxycinnamic acids to yield substituted benzoic acids.

Tissue cultures of *V. planifolia* were shown to produce 4-hydroxybenzaldehyde synthase, a constitutively expressed enzyme catalyzing chain formation of a hydroxycinnamic acid, believed to be the first reaction specific for formation of vanilla flavour compounds (Podstolski *et al*. 2002; Pak *et al*. 2004). Other studies (Havkin-Frenkel *et al*. 1996) accumulated 4-coumaric acid, 4-hydroxybenzaldehyde, 4-hydroxybenzyl alcohol, 3,4-dihydroxy-benzaldehyde, 4-hydroxy-3-methoxybenzyl alcohol, and vanillin. A cDNA that encodes a multifunctional methyltransferase that catalyzes the conversion of 3,4-dihydroxybenzaldehyde to vanillin holds promise for genetic transformation and engineering strategies for the synthesis of natural vanillin from alternate sources (Pak *et al*. 2004).

Floral fragrance and pollinator attractants

Floral fragrance of orchids has more than aesthetic value: the capacity to attract specific insect pollinators ensures the direct preservation of the species. The flower fragrances of three intraspecific taxa of *Cypripedium calceolus* were isolated by sorption on synthetic polymers and analysed by GC-MS, each with very distinctive fragrances, composed mainly of fatty acid derivatives, isoprenoids, and phenyl derivatives (Bergström *et al*. 1992). Earlier GC-MS analyses of floral fragrances of *Neofinetia* and *Vandofinetia* indicated that methyl benzoate, β-caryophyllene, linalool and α-terpinene contributed to the typical fragrance of flowers (Holman and Heimermann 1973). Using a novel headspace solid phase microextraction (HS-SPME) protocol, proposed for the analysis of floral scent, was used to identify mainly sesquiterpenes (C15 compounds) from *Orchis pallens* (Bartak *et al*. 2003), while a more rudimentary headspace contraption allowed for the identification of linalool, nerolidol (Matile and Altenburger 1988), benzaldehyde and caryophyllene (Altenburger and Matile 1990) rhythmical, diurnal emissions from *Odontoglossum* flowers. Musky notes of 16-hexadecanolide and (Z)-7-hexadecen-16-olide from *Epicattleya kyoguchi* and *Epidendrum aromaticum* were discovered in their headspace extracts, although the main volatile components were α-bergamotene in *E. aromaticum* and limonene in *C. aurantiaca* (Hirose *et al*. 1999). Floral fragrance composition of *Stanhopea* reveals primarily of mixtures of monoterpenes and simple aromatic compounds, the main being cineole, while sesquiterpenes, aliphatic acetates and aliphatic aldehydes are uncommon (Whitten and Williams 1992), the major fragrance component of *Stanhopea pulla* being *trans*-limonene oxide (Hills 1989). A huge number of orchids have been analysed for their floral scents (Kaiser 1993). Patt *et al*. (1988) found that lilac aldehydes and alcohols, and other monoterpene alcohols and hydrocarbons, α-pinene being the major constituent, aromatic aldehydes and alcohols were present in the charcoal or tenax adsorbed floral fragrance of *Platanthera stricta* (Patt *et al*. 1988).

Ipsdienol is a major component of the floral fragrance of several species of orchid, and the one aroid that is pollinated by male *Euglossine* bees (Whitten *et al*. 1988). In *Calanthe* headspace extracts, (*E*)-β-ocimene and linalol were the two main components of *C. izu-insu*, while for *C. sieboldii* they were (*E*)-cinnamic aldehyde, β-caryophyllene, linalol and methyl benzoate; in *C. izu-insu* sensory analysis showed that linalol and indole provided the fresh daphne and lily-of-the-valley like odours, whereas in *C. sieboldii* methyl benzoate, methyl salicylate, carvone and cinnamic aldehyde contributed to the sweet orange-like spicy odour; hexane extracts of both *Calanthe* species identified methyl hexadecanoate (13.5%) as the main compound (Awano *et al*. 1997). In *Dendrobium superbum*, mostly methyl ketones and 2-alkyl acetates may contribute to the attraction of the pollinator, the male melon fly (Flath and Ohinata 1982) while methyl cinnamate results in pollinator attraction to

Stanhopea (Williams and Whitten 1982) and *Gongora quinquenervis* (Williams *et al*. 1985).

Flower colour

Flavonoids are present in all higher plants, and in their structurally related forms, such as anthocyanins, flavones, and isoflavonoids, they serve important functions as flower and fruit pigments, UV-absorbing compounds or phytoalexins. The accumulation of flavone, flavonol and isoflavonoid compounds in response to UV light and pathogen stress has also been shown due to an increase in the rate of the *CHS* (chalcone synthase) gene transcription, whose cDNA clone was isolated from *Bromheadia finlaysoniana* (Liew *et al*. 1998a, 1998b). The anthocyanin content of some orchid genera have been thoroughly investigated (Strack *et al*. 1989). Yellow-flowered *Dendrobium* contain various kinds of carotenoids, viz. neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, and β-carotene (Thammasiri *et al*. 1986), while orange *Cattleya aurantiaca* contain the hydrocarbons β-carotene, γ-carotene, and lycopenes, and the monols β-cryptoxanthin and rubixanthin, as well as a small amount of xanthophylls (Matsui 1994). Seven anthocyanins (chrysanthemin, cyanin, seranin, ophrysanin, orchicyanin I/II, serapianin) were isolated from *Dactylorhiza*, *Nigritella*, *Orchis*, *Ophrys*, and *Serapias*, which also occur widely among other orchids (Strack *et al*. 1989). In *Dracula* (Pleurothallidinae) colours range from shades of white through shades of yellow, pink, blood red to dark maroon – almost black, and 5 anthocyanins accounted for 78% and 28% of the total anthocyanin content of *Dracula chimaera* and *D. cordobae*, respectively (Fossen and Øvstedal 2003). Acylated cyanidin glycosides/glucosides are the major anthocyanin contributors to the purple-red colour of *Bletilla striata* (Saito *et al*. 1995), *Laeliocattleya*, *Laelia*, and *Cattleya* (Tatsuzawa *et al*. 1996) flowers.

Other secondary metabolites

Organic compounds that are either specific to the plant family or xenobiotic can be transformed in tissue culture. Many orchid species produce secondary metabolites which are either isoprenoid compounds, including sterols (Hills *et al*. 1968; Wan *et al*. 1971), or derivatives of shikimic acid. Tissue cultures of *Cymbidium* 'Saint Pierre', *Dendrobium phalaenopsis*, *Epidendrum ochraceum* maintained *in vitro* on media used for other orchids transformed some isoprenoids (Kukułczanka and Wojciechowska 1983; Kukułczanka 1985; Mironowicz *et al*. 1987), primarily the hydrolysis of (±)-menthyl acetate to menthol (75-85%), the hydrolyzation of phenol acetates, aromatic-aliphatic alcohols and acatates of racemic aromatic-aliphatic alcohols (Mironowicz *et al*. 1993). The biological activity of *Cattleya in vitro*-derived phenolics was tested (Ishii *et al*. 1976).

Anoectochilus formosanus, a precious Chinese herb can be used wild or from tissue culture to treat cardiovascular disease (Huang *et al*. 1991).

Geodorum densiflorum, commonly known as the ground gem orchid, is a terrestrial herb distributed in india, Nepal and Bhutan and extended further in Southeast Asia, Papua New Guinea and Australia. This species is important for the anti-diabetic property of its underground pseudobulb (Hegde 1996), but due to its unrestricted collection, has become a rare plant, resulting in the development of *in vitro* micropropagation techniques (Roy and Banerjee 2002).

Habenariol, an uncommon ester (bis-*p*-hydroxybenzyl-2-isobutylmalate) and a freshwater feeding deterrant (of freshwater crayfish *Procambarus clarkii*) from the aquatic orchid *Habenaria repens* is closely related to a bis-ester glycoside and 2-[1-methyl-propyl]malate ester isolated from the non-aquatic orchid *Galeola faberi* (Li *et al*. 1993; Wilson *et al*. 1999). Habenariol is also an antioxidant, and being a phenolic substance, typically has this quality enabling it to neutralize free radicals, by inhibiting lipid peroxidation of human low density lipoprotein (LDL), a widely accepted model for determining antioxidant activity (Johnson *et al*. 1999), lower than α-tocopherol, but similar to carnosol and rosmarinic acid from rosemary, mangostin from *Garcinia mangostana*, catechins from tea, and anthocyanins and related polyphenols in grape juice or red wine (Shahidi *et al*. 1992).

The isolation of a large number of compounds from a series of Indian orchids (*Agrostophyllum brevipes*, *A. callosum*, *A. khasiyanum*, *Arundina bambusifolia*, *Bulbophyllum triste*, *B. reptans*, *Cirrhopetalum elatum*, *C. andersonii*, *C. maculosum*, *Coelogyne flaccida*, *C. cristata*, *C. uniflora*, *Cymbidium pendulum*, *Dendrobium amoenum*, *D. crepidatum*, *Eria flava*, *E. confusa*, *Lusia indivisa*, *L. volucris*, *Pholidota rubra*, *P. imbricata*, *Thunia alba*) by Majumder and colleagues encompassed a wide variety of stilbenoids (Majumder *et al*. 1996a, 1996b, 1998, 1999a, 1999b, 1999c, 2001), viz. stilbene (Majumder *et al*. 1998b), callosumin and callosuminin (Majumder *et al*. 1996b), cirrhopetalnthridin and cirrhopetalidinin (Majumder and Basak 1991b), imbricatin (Majumder and Sarkar 1982), flaccidin and flaccidinin (Majumder and Maiti 1988, 1989), isoarundinin I/II (Majumder and Ghosal 1994), callosinin (Majumder *et al*. 1995a), agrostophyllin (Majumder and Sabzabadi 1988), 6-methoxycoelonin (Juneja *et al*. 1987), flavanthrinin (Majumder and Banerjee 1990), and nudol (Stermiz *et al*. 1983; Bhandari *et al*. 1985); bibenzyls and bibenzyl derivatives, e.g. cirrhopetalidin, cirropetalinin, and crepidatin A (Majumder and Chatterjee 1989; Majumder and Basak 1991a; Majumder *et al*. 1999a), phenanthrenes and phenanthraquinones, e.g. bulbophyllanthrone and ochrone A,B (Bhaskar *et al*. 1991; Majumder and Sen 1991a, 1991b) and 9,10-dihydrophenanthrenes (Majumder *et al*. 1996) and their dimers (Majumder and Banerjee 1988; Majumder *et al*. 1997, 1998a, 1999b), phenanthropyrans and pyrones (Majumder and Sabzabadi 1988; Majumder and Maiti 1991) and their 9,10-dihydro derivatives (Majumder *et al*. 1982, 1999c, 2001), fluorenone (Majumder and Chakraborty 1989) and a few other polyphenolics (Majumder *et al*. 1994, 1995b), several triterpenoids (Majumder and Ghosal 1991), steroids of biogenetic importance (Majumder and Pal 1990) and some simple aromatic compounds (Majumder and Lahiri 1989). Phenanthrene derivatives have been found to be potent phytoalexins, while others act as endogenous plant growth regulators (Gorham 1980; Majumder *et al*. 2001). Convallarioides nudol, eranthridin, sitosterol, erianol were isolated from *Eria convallarioides* (Majumder and Kar 1989). β-Sitosterol, betulinic acid and some perfumery constituents were isolated from *Luisia indivisa* (Majumder and Lahiri 1989).

Flavone *C*-glycosides and flavonols were the most common constituents found in 53 and 37%, respectively of 142 species (75 genera) leaves (Williams 1979).

Bulbophyllanthrone: a cytotoxic phenanthraquinone from *Earina autumnalis* (Hinkley and Lorimer 1999).

The addition of glyphosate (as RoundUp®) resulted in the production of orchinol, a phenolic compound, in *Orchis morio* liquid culture (Beyrle *et al*. 1995).

Dihydrophenanthrenes and bibenzyl synthase are produced in the rhizomes of orchids after wounding, their induced formation depending on wounding and the extent of fungal infection (Gehlert and Kindl 1991).

A mannose-specific lectin was isolated from *Listera ovata* (van Damme *et al*. 1987).

CONCLUSIONS AND FUTURE PERSPECTIVES

This review provides an overview of what the author considers to be the most significant literature in orchid biotechnology and related sciences until about 2004-2006. Although other reviews have more recently been written on different aspects of orchids [historical aspects of propagation; Yam and Arditti 2009), asymbiotic seed germination (Kauth *et al*. 2008), effect of explant type on *in vitro* culture (Chugh *et al*. 2009), genetic transformation (Teixeira da Silva *et al*. 2011) and thin cell layers (Teixeira da Silva

2013), this is the first review to abridge such a wide range of themes and topics about orchids within a single review. Reviews on more current (~2004-present) topics related to orchids are now currently being prepared and have been published elsewhere (e.g., Hossain *et al*. 2013).

ACKNOWLEDGEMENTS

The author wishes to thank Pham Thanh Van for assistance with the referencing style. The author thanks Prof. Michio Tanaka for access to copies of difficult-to-access literature.

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