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In Vitro Propagation of Orchids For Their Conservation: A Critical Review

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ABSTRACT

Orchids are the most pampered, gorgeous and peculiar plant with implausible range of diversity. Their magnificent flower with stunning colour, glamorous shape and long lasting features make them commercially important.Inspite of having spectacular advancement in *in-vitro* micropropagation of orchids, problems frequently encountered have been exudation of large quantity of phenolics, choice of appropriate explants, shortage of efficient methods for seed germination, and seedling death during inoculation. All orchids have been listed in Appendix II of CITES and some even have been included in Appendix I. Present review makes an effort to bring together some recent studies on orchids via seeds, rhizomes, shoots tip, internodes, pseudobulbs, PLBs, leaves, roots, nodes as explants . These reported protocols, after initial testing their reliableability and efficiency can possibly be used for large scale mass multiplication along with *ex vitro* establishment of rare, threatened and endangered orchids to meet the horticultural, floricultural market demand.

KEYWORDS: Protocorm like bodies, Orchids, Seed germination, Conservation, Mass propagation.

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ORCHIDS: A BRIEF INTRODUCTION

Orchid's flowers are one of the most beautiful, peculiar and fascinating creations of God. The term 'Orchid' derives its origin from the Greek word 'Orchis'; meaning testicles [1]. Owing to the shape of their pseudobulbs resembling testicles, these plants were named as orchids. The term orchid was first used by the famous Greek philosopher, Theophrastus (372-286 B.C) in his book "de Historia Plantarum". He also highlighted therapeutic significance of orchids in his book "Enquiry into Plants" [2] . The orchid family, Orchidaceae, is one of the largest, most evolved and diverse families of flowering plants. It comprises 17000 to 35000 species belonging to 750 to 850 genera [3]. About 1300 species are estimated to occur in India [3, 4]. The latest estimate in terms of the numerical strength of the members of this family is expected to fall somewhere close to 20,000 species [5]. A new estimate made for Appendix 2 of CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) is about 19,500 species [3].

Orchids are cosmopolitan in distribution, occurring almost in all habitats including glaciers and dry desert [6,7]. Their extraordinary diversity predominates in tropical and subtropical zones [6,4] . About 73% of orchids are epiphytes and rest are lithophytes, semi-terrestrial and true terrestrial [3]. They use other plants (trees) merely for support and space. Monopodial (single stem) and Sympodial (multiple stems) are the two major growth patterns found among orchids. Indeterminate growth of monopodial stem produces leaves but lacks rhizome [8]. Orchids belonging to this category are *Phalaenopsis*, *Vanda sp*., *Vanilla sp*. and so on [9,10]. The sympodial orchids possess storage organs known as pseudobulbs which act as reservoirs of food and water. This habit appears as successive growth, each originating from the base of preceding one, e.g. *Paphiopedium sp*., *Oncidium sp*., *Dendrobium sp*., *Cattleya sp*., *Cymbidium sp*., *Arundina sp*., *Phaius sp*. and *Anoectochilussp*.etc.[8,10].

Orchids reproduce by means of seeds with pods or capsules being the fruiting body, each of which contains millions of microscopic, which disperse like spores or dust particles, contain neither endosperm nor fully differentiated embryo[11-14]. Despite production of seeds in large numbers, the plants produced are limited because of the low survival rate of seeds and high rate of mortality of seedlings[12-16]. Cotyledon, radicle and plumule are almost absent except in few species, such as, *Sorbralea macrantha* and *Bletilla hyacynthina*, which have well differentiated embryos and rudimentary cotyledons [9.]

MEDICINAL ORCHIDS

A detailed literature survey has revealed that 209 species of orchids are used for the treatment of one or the other ailments afflicting human beings (Table 1). Tubers are the most commonly used organs for therapeutic purposes followed by whole plants, roots, pseudobulbs and rhizomes (Fig. 1).

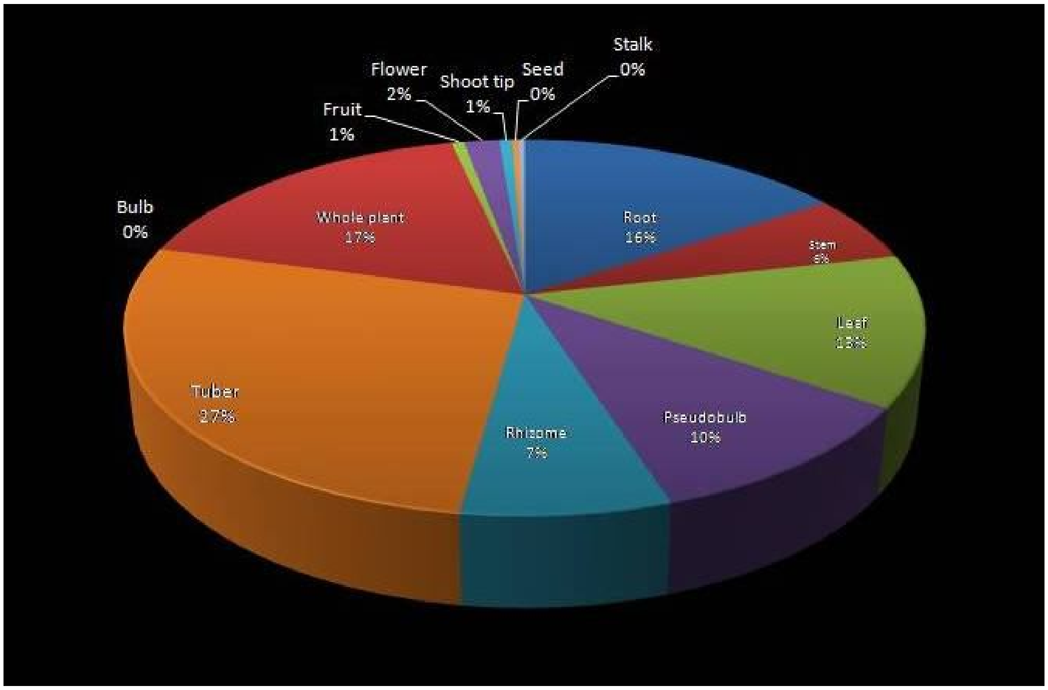

Figure 1: Parts of orchid plants used as herbals.

Table1 - List of orchids used for various medicinal purposes as per quoted literature.

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
1.	*Acampe carinata* (Griff.) Panigrahi.	Rasna (H), Small Warty Acampe (E)	Root, leaf	The root paste is used in scorpion and snake bite, rheumatism and uterine diseases.	17
2.	*Aerides crispum* Lindl	-	Whole plant	Cure ear pain and deafness	18
3.	*Acampe papillosa* (Lindl.) Lindl.	Small Warty Acampe (E)	Root	It is useful in poisonous infections, and fever	19
4.	*Acampe praemorsa* (Roxb.) Blatt. & McCann	Acampe Orchid (E)	Root	Root paste of *Acampe praemorsa* and *Asparagus recemosus* are taken empty stomach to cure arthritis.	20
5.	*Acampe wightiana* Lindl.	-	Root	The plant is used to make tonic and also useful in cold and cough.	21
6.	*Aerides multiflorum* Roxb.	-	Whole plant	It showed antibacterial activity against *Salmonella aureus* and *Klebsiella pneumonia.*	22
7.	*Aerides odorata*	-	Root, leaf	Reduces joint pain and	17

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
	Lour.			swelling. The leaf juice is taken to cure tuberculosis.	
8.	*Anoecetochilus formosanus* Hayata.	Jewel Orchid (E)	Tuber	It is useful in diabetes, fever and liver spleen disorder.	23,24
9.	*Anoectochilus roxburghii* (Wall.) Lindl.	Roxburgh's Anoectochilus (E)	Whole plant	Treatment of fever, pleurodynia, snake bike, lung and liver disease, hypertension.	25
10.	*Arundina graminifolia* (D.Don) Hochr.	Bamboo Orchid, Tapah Weed, Kinta Weed, Bird Orchid (H)	Rhizome	Root decoction is used as pain killer.	26
11.	*Bletilla formosana* (Hayata) Schltr.	The Beautiful Bletilla (E)	Tuber	Used for the treatment of lung, liver and stomach disorder.	23
12.	*Bletilla striata* (Thunb.) Rchb. f.	Chinese ground orchid, Hardy orchid	Tuber	Antibacterial and anti-inflammatory.	27,28
13.	*Bletia hyacinthina* (Wild) R.Br.	Hyacinth Orchid	Tuber	Beneficial in tuberculosis, cracked skin, ulcers and breast cancer.	29
14.	*Brachycortis obcordata* (Lindl.) Summerh.	Heart-Shaped Brachycorythis	Root	Use as tonic with milk, cure dysentery	30
15.	*Bulbophyllum careyanum* (Hook.) Sprengel	Carey's Bulbophyllum	Leaves, pseudobulb	Cause abortion, used in burns	-do-
16.	*Bulbophyllum cariniflorum* Rchb. F.	Keeled Flower Bulbophyllum (E)	Root	Induce abortion within 2-3 month of pregnancy	17
17.	*Bulbophyllum kwangtungense* Schltr.	The Kwangtung Bulbophyllum (E)	Tuber	Treat pulmonary Tuberculosis, reduce fever and promote the production of body liquid	31
18.	*Bulbophyllum leopardinum* (Wall.) Lindl.	The Leopard Spotted Bulbophyllum	Whole plant	Decoction used in burns	30
19.	*Bulbophyllum lilacinum* Ridl.	The Lilac Bulbophyllum (E)	Pseudobulbs	Fluid of pseudobulb with water keep the body fresh and remove tiredness.	4
20.	*Bulbophyllum odoratissimum* (Sm.) Lindl.	The Fragrant Bulbophyllum (E)	Whole plant	Treat tuberculosis, chronic inflammation and fracture.	32
21.	*Bulbophyllum neilgherrense*	The Nilgiri Mountain	Pseudobulbs	Pseudobulp juice restore	4

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
	Wight	Bulbophyllum (E)		youthness and act as antiageing medicine.	
22.	*Bulbophyllum umbellatum* Lindl.	The Umbrella Bulbophyllum	Whole plant	Enhance congenity	30
23.	*Calanthe discolor* Lindl.	Ground Orchid (E)	Whole plant	Hair restoring.	33
24.	*Calanthe liukiuensis* Schltr.	-	Whole plant	Hair restoring.	33
25.	*Calanthe plantaginea* Lindl.	The Plantago-Like Calanthe	Rhizome	Used as tonic and aphrodisiac	30
26.	*Calanthe puberula* Lindl.	The Hairy *Calanthe*	Rhizome	Used with milk as tonic	-do-
27.	*Calanthe sylvatica* (Thou) Lindl.	The Forest-Dwelling Calanthe	Flower	Cures nose bleeding	-do-
28.	*Calanthe tricarinata* Lindl	Monkey Orchid, Hardy Calanthe Orchid	Leaf, Pseudobulbs	Leaves decoction applied on sores and eczema. Pseudobulbs having aphrodisiac properties	-do-
29.	*Catasetum barbatum* Lindl.	The Bearded Catasetum (E)	Whole plant	Febrifuge, anti-inflammatory.	34
30.	*Cephalanceropsis gracillis* (Lindl.)	-	Whole plant	Suppress cancer	31
31.	*Cephalanthera longifolia* K. Fritsch	Narrow-*leaved Helleborine* or Sword-*leaved Helleborine*	Rhizome	Heals wounds. Used as appetizer and tonic.	30
32.	*Cleisostoma williamsonii* (Rchb.f.) Garay.	Williamson's Cleisostom (E)	Whole plant	Plant's paste is used as astringent.	19
33.	*Coelogyne corymbosa* Lindl.	The Umbrella-Like Coelogyne (E)	Pseudobulb	Pseudobulb juice used in healing wounds	35
34.	*Coelogyne cristata* Lindl.	Crested Coelogyne (E), Gondya (H)	Pseudobulb	Heals wounds	17
35.	*Coelogyne flaccida* Lindl.	The Loose Coelogyne	Pseudobulb	Useful in headache, fever and constipation	30
36.	*Coelogyne fuscescens* Lindl.	Ocher Yellow Coelogyne (E)	Pseudobulb	Treatment of stomachache.	36
37.	*Coelogyne gardneriana* Lindl.	Gardner's Neogyna (E)	Whole plant	Antibacterial against *klebsiella pneumoniae* and	37

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
				E.coli.	
38.	*Coelogyne nitida* (Wall. ex Lindl) D. Don.	The Shining Coelogyne	Pseudobulb	Paste is useful in fever, headache and burn.	30
39.	*Coelogyne ovalis* Lindl.	The Oval Coelogyne	Pseudobulb	Aphrodisiac	-do-
40.	*Coelogyne prolifera* Lindl.	Prolific Coelogyne, Yellowish Coelogyne	Pseudobulb	Relieve fever, headache and backache	-do-
41.	*Coelogyne stricta* (D. Don) Schltr	The Rigid Coelogyne	Pseudobulbs	Relieves fever and headache	-do-
42.	*Coelogyne viride* (L.)	-	Rhizome	Memory deficits.	38
43.	*Coelogyne punctulata* Lindl.	Spotted Coelogyne (E)	Pseudobulb	Pseudobulb powder is used in burn injury and healing wounds.	-do-
44.	*Conchidium muscicola* (Lindl.) Lindl.	-	Whole plant	Useful in repiratory, cardiac and nervous disorders	30
45.	*Corallorhiza maculate* Raf.	Spotted Coral Root (E)	Roots, stalks	Dried stem is used in making tea and treats pneumonia patients	-do-
46.	*Coryborkis veratrifolia* (Reinw.) Blume	White Cinnamon Orchid (E)	Leaf	Leaf juice is used to treat fever.	-do-
47.	*Cremastra appendiculata* (D.Don) Makino	-	Bulbs	It is associated with liver, spleen and stomach meridians. Fight tumors and skin lesions.	39
48.	*Crepidium acuminatum* (D. Don) Szlach	Jivak (H)	Rhizome, root, psudobulb	Treats weakness, fever, tuberculosis. and bronchitis.	30
49.	*Cymbidium devonianium* Lindl. ex Paxton	-	Whole plant	Treats cough and cold	-do-
50.	*Cymbidium elegans* Lindl.	The Elegant Cymbidium	Leaves, Pseudobulbs, roots	Used for healing wounds	-do-
51.	*Cymbidium iridioides* D. Don	The Iris-Like Cymbidium	Leaves, Pseudobulbs, roots	Used as tonic and stop bleeding.	-do-
52.	*Cymbidium goeringii* (Rchb.f.)	Hardy Cynbidium Orchid (E)	Whole plant	Shows diuretic activities.	40

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
53.	*Cymbidium macrorhizon* Lindl.	Large Root Cymbidium (E)	Rhizome	Used as diaphoretic and febrifuge.	41
54.	*Cymbidium aloifolium* (L) Sw.	Aloe Leaf Cymbidium (E)	Root, leaf, whole plant	Reduce paralysis.	17
55.	*Cymbidium ensifolium* (L) Sw.	Cymbidium With The Sword Shaped Leaves (E)	Rhizome	Decoction from rhizome cure gonorrhoea and eye sores.	19
56.	*Cymbidium giganteum* wall ex Lindl.	The Iris-Like Cymbidium (E)	Leaf	Leaf juice has blood clotting properties	42
57.	*Cymbidium longifolium* D.Don	-	Pseudobulb	Used as demulcent.	35
58.	*Cypripedium calceolus* L.	Yellow lady slipper (E)	Root, rhizome	Useful in headaches, diabetes, dysentery, paralysis etc.	43
59.	*Cypripedium cordigerum* D. Don	Heart-shaped Slipper Orchid	Roots	Used as tonic	30
60.	*Cypripedium elagans* Rchb.f.	Elegant Slipper Orchid (E)	Root	Used in epilepsy, rheumatism, madness and hysteria.	19
61.	*Cypripedium himalaicum* (Rolfe) Kranzl.	Himalayan Slipper Orchid	Whole plant	Cures heart,chest, stone and urinary disorders.	30
62.	*Cypripedium macranthos*	The Large Flowered *Cypripedium* (E)	Rhizome	Used for skin diseases.	44
63.	*Cypripedium parviflorum* Salisbury	Lesser Yellow Lady's Slipper, Smaller Yellow Lady's Slipper (E)	Rhizome	Treats various disorders like anxiety, fever, headache, tension, insomnia, pain of menstruation and child birth.	45
64.	*Cypripedium pubescens* Wild.	Greater yellow lady's slipper (E)	Root	It is used in diarrhea, dysentery, malnutrition, diabetes, impotence, headache.	6
65.	*Dactylorhiza hatagirea* (D.Don) Soo.	Hathajari, Hathpanja (H)	Root, rhizome	Tubers used in tonic and aphrodisiac.	2
66.	*Dactylorhiza maculate* (L.) Soo	The Spotted Dactylorhiza (E)	Tuber	The plant has aphrodisiac properties.	46
67.	*Dactylorhiza purpurella* (Stephen. & Stephen.) Soo.	Northern Marsh Orchid (E)	Tuber	It has antiageing and aphrodisiac properties.	-do-
68.	*Dendrobium alpestre*	The Mountain Living	Pseudobulb	Used to treat pimple and	41

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
	Royle	Dendrobium (E)		other skin problem.	
69.	*Dendrobium amoenum* Wall. ex Lindl.	The Lovely Dendrobium (E)	Leaf	Skin diseases	47
70.	*Dendrobium aurantiacum* Rchb.f.	The Yellow Flowered Dendrobium (E)	Leaf	Diabetes	48
71.	*Dendrobium candidum* Wall. ex Lindl.	The White Dendrobium (E)	Leaf	Diabetes	49
72.	*Dendrobium crepidatum* Griff.	The Shoe-lipped Dendrobium	Pseudobulb	Paste applied in dislocation and fracture of the bone.	30
73.	*Dendrobium chrysanthum* Wall.	Golden Flowered Dendrobium (E)	Leaf	Antipyretic, eyes-benefiting, immunoregulatory purposes, skin diseases	50
74.	*Dendrobium crumenatum* Sw.	The Pigeon Orchid (E)	Leaf	Beneficial in pimples	19
75.	*Dendrobium densifiorum* Lindl. ex Wall.	The Densely Flowered Dendrobium (E)	Leaf	Promotes the production of body fluid.	51
76.	*Dendrobium densiflorum* Lindl.	Pineapple Orchid	Psudobulbs	Remove pimples and cures other skin problem.	30
77.	*Dendrobium eriaeflorum* Griff.	The Eria-Like Flowered *Dendrobium*	Psudobulbs	Used as tonic and even applied in bone fracture.	-do-
78.	*Dendrobium farmeri* Paxton	Farmer's Dendrobium (E)	Whole plant	Antibacterial activity against *Klebsiella pneumoniae,E.coli* and *Salmonella typhi.*	37
79.	*Dendrobium fimbriatum* Hook.	The Fringe-Lipped Dendrobium (E)	Leaf	Leaf paste applied on fracturated area to set bone.	52
80.	*Dendrobium heterocarpum* Wall.ex Lindl.	Noble Dendrobium	Psudobulbs	Paste applied in dislocation and fracture of the bone.	30
81.	*Dendrobium herbaceum* Lindl.	Grassy Dendrobium (E)	Leaf	Paste of fresh leaves applied on wound and treats syphills.	17
82.	*Dendrobium loddigesii* Rdfe.	Loddiges' Dendrobium (E)	Leaf	Used as tonic to nourish the stomach, replenish body fluid, and reduce fever along wtih anticancer properties.	53
83.	*Dendrobium longicornu* Lindl.	-	Whole plant	Relieve cough and fever	30

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
84.	*Dendrobium macraei* Auct.	Jivanti(H)	Tubers	Used in tonic preparation.	54
85.	*Dendrobium macrostachyum* Lind.	Leafless Dendrobium (E)	Tender shoot tip	Tender tip juice is used as an ear drops for earache.	55
86.	*Dendrobium monoiliforme*	The Necklace-Shaped Dendrobium (E)	Stems	Tonic, antipyretic, aphrodisiac, analgesic.	56
87.	*Dendrobium monticola* Hunt & Stummerh.	The Mountain Living Dendrobium (E)	Pseudobulb	Useful in pimples and skin eruption.	19
88.	*Dendrobium moschtum* Lindl.	The Musky-smelling Dendrobium	Pseudobulb	Treat dislocated and fractured bones	30
89.	*Dendrobium nobile* Lindl.	Noble Dendrobium(E)	Pseudobulb seed	Plant is used in the treatment of pulmonary tuberculosis, dyspepsia,fever and anorexia.	57,58
90.	*Dendrobium normale* Falc.	-	Whole plant	Entire plant have aphrodisiac and tonic properties.	4
91.	*Dendrobium ovatum* (Wild.) Kranzl.	Green Lipped *Dendrobium (E)*	Whole plant	Useful in stomachache and constipation.	19
92.	*Dendrobium pumiluim* Roxb.	The Broad-Stemmed Dendrobium (E)	Whole plant	The Malays use it as a drug in dropsy.	59
93.	*Dendrobium tosaense* Makino	-	Leaf	Treatment of anxiety and panic.	60
94.	*Dendrobium transparens* Wall. ex Lindl.	The Translucent Dendrobiu	Pseudobulb	Treat dislocated and fractured bones	30
95.	*Desmotrichum fimbriatum* Blume	-	Whole plant	Cure disorder of bile, blood and phlegm. Helpful in treatment of snake bite, scorpion sting and even used as tonic in debility due to seminal losses.	59
96.	*Dienia cylindrostycha* Lindl.		Pseudobulb	Used as tonic	30
97.	*Ephemerantha lonchophylla* (Hook . F.) Hunt & Summerch	-	Stems	It is used as tonic to nourish the stomach, promote the production of body fluid, and reduce fever.	61
98.	*Epipactis helleborine* (L.) Crantz.	Bastard Hellebore, Broadleaf Helleborine, Common Helleborine	Root, rhizome	Roots of this plant are medicinal which cure insanity.	2

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
		(E)			
99.	*Epipactis latifolia* Wall.	Broad-helleborine, Helleborine orchid (E)	Rhizome	Rhizome is regarded as aphrodisiac and is used to treat nervous disorder. Infusion of leaves is useful in case of intermittent fever.	4
100.	*Epidendrum mosenii* Barb. Rodr.	-	Stem	Analgesic activity	52
101.	*Epidendrum rigidum* Jacq.	Rigid star orchid (E)	Stem	Replenish body fluid	63
102.	*Eria bambusifolia* Lindl.	The Bamboo-Like Leafed Eria (E)	Whole plant	The plant is used to cure hyper acidity and other stomach disorders	64
103.	*Eria muscicola* (Lindl.) Lindl.	The Moss-Growing Eria (E)	Pseudobulb	It is used to treat chest, heart, lung, eye, ear and mental problems	65
104.	*Eria pannea* Lindl.	The Flag Eria (E)	Root, leaf	Decoction of leaves and roots are used in boneache.	19
105.	*Eria spicata* (D.Don.) Hand Mazz	The Spicate Eria (E)	Stem	Stem paste is used to cure headache and stomach disorder.	35
106.	*Eria pubescens* Lindl.	-		Fluids extracted from pseudobulb mixed with water and taken to increase the sexual strength.	4
107.	*Eulophia campestris* Wall.	Salep (E), Salam (H)	Tuber	Blood purifier, demulcent anthemnitic	59
108.	*Eulophia dabia* (D.Don) Hochr.	Salep misri (H) Salam misiri (H)	Tuber	Tubers used in stomach tonic, aphrodisiac and blood purifier during heart problem.	2
109.	*Eulophia epidendraea* (J.König ex Retz.) C.E.C.Fisch.	The Epidendrum-Like Eulophia (E)	Tuber	The tubers are used as vermifuge and to treat anorexia and anthrax.	19
110.	*Eulophia graminea* Lindl.	Grass Eulophia (E)	Tuber	Tuber's decoction are used as vermifuge.	66
111.	*Eulophia herbacea* Lindl.	-	Tuber	It is supposed to be a kind of salep and tonic.	19

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
112.	*Eulophia nuda* L.	Whitton root, Salep (E)	Tuber	Demulcent, anthemnintic and helpful in cardiovascular diseases.	59
113.	*Eulophia ochreata* Lindl.	Golden-Yellow Eulophia (E)	Tuber	Useful in male sterility, sexual impotency, vigour and to some extent show aphrodisiac properties.	67
114.	*Eulophia pratensis* Lindl.	The Spectacular Eulophia(E)	Tuber	Tubers are used externally as well as internally to remove scrofulous gland in the neck.	59
115.	*Eulophia spectabilis* (Dnnst.) Suesh	The Spectacular Eulophia (E)	Leaf	Aphrodisiac and leaf decoction is also used against vermifuge.	17
116.	*Flickingeria fugax* (Rchb. f.) Seidenf.		Whole plant	Used as tonic	30
117.	*Flichingeria macraei* (Lindl.) Sidenf.	Macrae's Flickingeria (E)	Pseudobulb	Extracted juice of pseudobulb should be taken twice a day for 21 days to cure skin allergy and even applied on an affected part to cure eczema.	17
118.	*Galeola foliate* (F.Muell.)	Great Climbing Orchid (E)	Stems	Treatment of some infections	68
119.	*Galeris strachaeyi* (Hook. f.) P. F. Hunt	-	Tuber	Cure headache and even used as tonic	30
120.	*Gastrodia elata* Blume.	Gastrodia (E)	Whole plant	Treatment of convulsive diseases such as epilepsy	29
121.	*Gastrodia orobanchoides* (Flac.) Benth.	-	Tuber	Tubers are edible and sweet in taste .	4
122.	*Geodorum densiflorum* (Lam.) Schltr.	Nodding Swamp Orchid, Shepherds Crook Orchid (E)	Root, tuber	Fresh root paste with honey regulate menstrual cycle, snake bite, cuts and wounds.	17
123.	*Geodorum recurvum* (Roxb.) Alston	The Bent Geodorum (E)	Tuber	Decoction of dried tuber are used to cure malaria and suppress tumors.	-do-
124.	*Goodyera repen* (L.) R.Br.	Creeping Rattlesnake Plantain, Lesser Rattlesnake Plantain		Cures appetite, stomachache, cold, kidney, stomach disorder.	4

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
		(E)			
125.	*Goodyera schlechtendaliana* Rchb. f.	-	Whole plant	Tonic for internal injuries and to improve circulation.	69
126.	*Gymnadenia conopsea* (L.) R.Br.	Fragrant Orchid (E)	Tuber	It is used as aphrodisiac	70
127.	*Gymnadenia orchidis* Lindl.	Himalayan Fragrant Orchid	Roots, Pseudobulbs	Heals wound and even used in urinary and liver disorder	30
128.	*Habenaria acuminata* Lindl.	The Acuminate Habenaria(E)	Tuber	The tubers are used as tonic.	2
129.	*Habenaria commelinifolia* (Roxb.) Wall.ex Lindl.	Commelina-Leaf Habenaria(E)	Root	6-8 drops of this roots decoction administered orally on an empty stomach for 10 days to cure spermatorrhea and urinary trouble.	17
130.	*Habenaria crinifera* Lindl.	The Hair Carrying Habenaria (E)	Tuber	Cure headache	71
131.	*Habenaria edgeworthii* Hook. F.ex Collett.	Vridhi (H) Edgeworth's Habenaria (E)	Tuber	Rejuvenator, spermopiotic and even regarded as tonic and blood purifier.	2,72
132.	*Habenaria foliosa* (Sw.) Rchb. f	The Leafy Habenaria (E)	Tuber	Plant is used medicinally by the Zulus (Africa).	21
133.	*Habenaria furcifera* Lindl.	The Fork-Carrying Habenaria (E)	Tuber	Ointment for cuts, wounds and poisonous bites.	55
134.	*Habenaria goodyeroides* D.Don	The Goodyera-Like Peristylus (E)	Tuber	The tuber is used as tonic.	2
135.	*Habenaria griffithii* Hook.f.(D.Don)	Griffith's Diphyllax (E)	Tuber	The tuber is used as tonic.	-do-
136.	*Habenaria hollandiana* Sant.	-	Tuber	Fresh plant paste is aaplied externally for scorpion sting and for infested sores.	55
137.	*Habenaria intermedia* D.Don	Ridhi (H) Intermediate Habenaia (E)	Tuber	Cooling, spermopiotic, rejuvenator and even used as tonic, cure various blood diseases.	2,72
138.	*Habenaria longicorniculata* Graham	The Small Horned Habenaria (E)	Tuber	Tubers decocotion with turmeric powder applied externally on affected part to	17

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
				cure leucoderma.	
139.	*Habenaria marginata* Coleb.	Golden Yellow *Habenaria (E)*	Tuber	Treatment of malignant ulcer .	-do-
140.	*Habenaria pectinata* D.Don	Comb *Habenaria* (E)	Leaf, tuber	The leaves are crushed and applied in case of snake bites. Tubers mixed with condiments are used in arthritis.	72
141.	*Habenaria plantaginea* Lindl.	The Platago-Like Habenaria (E)	Tuber	Tablet made form tuber paste cure chest pain and stomachache.	55
142.	*Habenaria repens* Nutt.	Water Spider Orchid (E)	Tuber	It is used as aphrodisiac	72
143.	*Habenaria roxburghii* Nicolson	Roxburgh's *Habenaria* (E)	Tuber	Decoction from tuber applied externally to cure snake bite.	55
144.	*Herminium lanceum* (Thunb.ex Sw.)	Jalya (H)	Stem, leaves	Cures diabetes, fever, bleeding and urinal disorders.	74,75
145.	*Herminium monorchis* (Linn.) R .Br.	The musk orchid	Roots	Tonic	30
146.	*Hetaeria oblique* Blume	The Deviating *Hetaeria* (E)	Whole plant	The plant is used by Malaya for poulticing sores.	76
147.	*Liparis nervosa* (Thunb) Lindl.	-	Tubers	Cure malignant ulcers and stomachache	
148.	*Listera ovate* (L.) R. Br.	Common Twayblade (E)	Tubers	Used to treat Stomach diseases and bowel irritation.	77
149.	*Lusia tenuifolia* Blume	-	Rhizome	The rhizomes and leaves are used as an emollient..	78
150.	*Lusia trichorhiza* (Hook.) Blume	-	Root	Paste of dried plant cure jaundice, reduce muscular pains and even antidiarrhoea (for cattle).	17
151.	*Luisia zeylanica* Lindl.	-	Stem	Stem is used in burns and to treat fractures.	59
152.	*Malaxis acuminta* D.Don	Rishbhak (H)	Rhizome, pseudobulb	Used as tonic to cure tuberculosis, fever and enhance sperm production. It is important ingredient of Chayvanprash of 'Asthavarga'	2,72

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
				group of drug.	
153.	*Malaxis cylindrostachya* (Lindl.) Kuntze	Adder Mouth Orchid (E)	Pseudobulb	Pseudobulb preparation is considered as tonic	2
154.	*Maxillaria densa* Lindl.	The Crowded *Maxillaria (E)*	Whole plant	Painkiller	79
155.	*Malaxis mucifera* (Lindl.) Kuntze	Jeevak (H), Adder Mouth Orchid (E)	Pseudobulb	It is important ingredient of 'Asthavarga', used as tonic, rejuvenating drug and cure fever, phthisis.	2
156.	*Malaxis rheedii* Sw.	The Resupinate Malaxis (E)	Tuber	Paste of tuber is useful in case of insect bite and rheumatism.	55
157.	*Malaxis wallichii* (Lindl.) Deb	The Gradually Tapering Malaxis (E)	Pseudobulb	It is said to cure tuberculosis and enhance sperm formation.	4
158.	*Microstylis mucifera* (Linn.) Ridl.	-	Root	Roots of plant promote sperm formation.	-do-
159.	*Neottianthe calcicola* (W.W. Sm.) Soo.	-	Rhizome	Tonic	30
160.	*Nervilia aragoana* Guad.	Aragoa-Like Nervilia (H), Sthalapadma (H)	Leaf, tuber	Leaves and tuber paste is used as ointment and medicine after childbirth	55
161.	*Nervillia plicata* (Andr.) Schltr.	Pleated Leaf Nervillia (E)	Tuber	Tuber paste is used as an external application for insect bites.	-do-
162.	*Nidema boothii* (Lindl.) Schltr.	Booth's *Nidema* (E)	Whole plant	Relaxant agent	63
163.	*Oberonia anceps* Lindl.	-	Leaf	The malayas use the leaves for poulticing.	76
164.	*Oberonia caulescens* Lindl.	-	Tubers	Useful in lever disorders.	30
165.	*Oberonia pachyrachis* Rchb.f.ex Hook.f.	Thick Spine Oberonia (E)	Leaf	Antibacterial	37
166.	*Oberonia wightiana* Lindl.	-	Leaf	Leaf is crushed and taken as medicine to suppress tumour.	55
167.	*Orchis latifolia* L.	Salep orchid (E)	Tuber	Tuber powder is added in milk or water to treat chest irritation, diarrhoea and	21

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
				chronic dysentery	
168.	*Orchis laxiflora* Lam.	Salab mishri (H)	Bulb	It is used to cure diarrhea, bronchitis and convalescence.	6
169.	*Otochilus albus* Lindl.	-	Whole plant	Tonic	30
170.	*Otochilus lancifolius* Griff.	-	Pseudobulb	Treat dislocated and fractured bones	-do-
171.	*Otochilus porrectus* Lindl.	-	Whole plant	Treats sinusitis rheumatism and even used as tonic	-do-
172.	*Paphiopedilum insigne* (Lindl.) Pfitz.	Slipper orchid or Venus Slipper orchid (E)	Whole plant	Effective against amoeboid dysentery.	36
173.	*Papilionanthe teres* (Roxb.) Schltr.		Whole plant	Paste is useful to treat dislocated bones	30
174.	*Pecteilis susannae* (L.) Rafin.	Susanna's Pecteilis (E)	Tuber	Tubers are used in boils.	2
175.	*Peristylus lawii* Wight	-	Tuber	Useful in case of insect bites.	55
176.	*Phaius tankervilliae* (Alt.) Blume	Nun's orchid (E)	Pseudobulb, whole plant	Heals swelling, treats dysentery and act as pain killer.	-do-
177.	*Pholidota chinensis* Lindl.	The Chinese Pholidota(E)	Pseudobulbs	Is taken for scrofula, feverish stomachache, toothache, chronic bronchitis, and duodenal ulcer.	80
178.	*Pholidota articulata* Lindl.	Harjojan (H), The Articulated Pholidota (E)	Whole plant	Tonic, antibacterial, bone fracture, skin diseases.	2
179.	*Pholidota imbricata* (Roxb.) Lindl.	Rattlesnake Orchid (E)	Whole plant	The plant is used as tonic, cure abdominal pain, rheumatism and even heals bone fractures.	35
180.	*Pholidota pallida* Lindl.	The Pale Pholidota (E)	Pseudobulb	Extracted juice from pseudobulb is applied on cut as haemostate.	81
181.	*Platanthera chlorantha*	The Greater	Rhizome	Plant is used to make	46

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
	(Custer) Rchb.	Butterfly-orchid (E)		ointment and applied to ulcers.	
182.	*Platanthera sikkimensis* (Hook. f.) Kraenzlin.	-	Bulbs, Pseudobulb	Relieve rheumatic and abdominial pain	-do-
183.	*Pleione humilis* (Sm.) D. Don	Ground Growing Pleione	Pseudobulb	Heals wound and used as tonic	-do-
184.	*Pleione maculate* (Lindl.) Lindl.	The Spotted Pleione (E)	Rhizome	Used in stomach and liver disorder	35
185.	*Pleione praecox* (Sm.) D. Don		Pseudobulb	Used in healing of wound and used as tonic	30
186.	*Polystachya* concreta (Jacq.) Garay & Sweet.	Pale Flowered Polystachya (E)	Tuber	Decoction of tuber with honey is useful for treatment of arthritis.	17
187.	*Rhynchostylis retusa* (L.) Blume	Banda, Rasna (H), Foxtail Orchid (E)	Leaf, whole plant	Antibacterial and cure rheumatic disease.	2
188.	*Saccolabium papillosum* Lindl.	-	Root	It has cooling effect and specific for rheumatism.	21
189.	*Satyrium nepalense* D.Don.	Salam misiri, Banalu (H)	Tuber, root	The dried tubers are used in tonic and also in malaria and dysentery	2
190.	*Scaphyglottis livida* Schltr.	The Bluish *Scaphyglottis (E)*	Whole plant	Analgesic agent and anti-inflammatory.	79
191.	*Seidenfia rheedii* (sw.) Szlach.	The Resupinate Malaxis (E)	Root	Roots decoction with honey cure cholera	17
192.	*Smitinandia micrantha* (Lindl.) Holttum	-	Whole plant	Tonic and antibacterial	30
193.	*Spiranthes australis* (R.Brown) Lindl.	Pink Spiral Orchid (E)	Whole plant	Treatment of bacterial and inflammatory diseases, cancer, blood and chest disorder.	82
194.	*Spiranthes mauritianum* Scop.	-	Whole plant	Used for snake bites and scorpion stings.	83
195.	*Spiranthes sinensis* (Pers.)	Austral Ladies Tresses (E)	Roots	Aphrodisiac, treatment of hemoptysis, epistaxis, headache, chronic dysentery and meningitis.	84,85
196.	*Taprobanea spathulata* (L.) Sperg.	The Spathulate Vanda (E)	Flower, whole plant	Tuber’s decoction cure asthma and mania.	19

S. No.	Botanical Name	Common Name (E:English, H:Hindi)	Part(s) Used	Ethnomedical Uses	Reference(s)
197.	*Thunia alba* (Lindl.) Rchb. F.		Whole plant	Useful in treating dislocated bones.	30
198.	*Tropidia curculigoides* Lindl.	The Curculigo-Like Tropida (E)	Root	Cure diarrhoea and malaria.	86
199.	*Vanda coerulea* Griff.ex Lindl.	Autumn lady's tresses orchid, blue vanda (E)	Flower	Effective against glaucoma and blindness.	87
200.	*Vanda cristata* Wall. Ex Lindl.	The Comb Vanda(E)	Leaf	Antibacterial and used as expectorant	88
201.	*Vanda parviflora* Lindl.	The Small Flowered Vanda(E)	Leaf , root	Antiviral, anticancerous and treats nervous disorder, rheumatism etc.	89
202.	*Vanda roxburghii* R.Br.	Vandae (E)	Leaf, roots	Brings down fever, treatment of otitis, dyspepsia and rheumatism.	90
203.	*Vanda spathulata* (L.) Spreng.	The Spathulate Vanda (E)	Flower	Flower's Powder are used in the treatment of asthma and mania	91
204.	*Vanda tessellata* (Roxb.) Hook. ex G. Don.	Vanda (E)	Roots, leaves and flowers	Aphrodisiac, analgesic, nervine tonic and used in sexually transmitted diseases, fever, rheumatism .	17, 72, 86, 92
205.	*Vanda testacea* (Lindl.) Rchb.f.	The Brick-Red Vanda (E)	Root	Cure earache, asthma and bone fracture of cattle	17
206.	*Vaniila griffithii* Rchb.f.	Griffith's Vanilla (E)	Leaf, stem	It provides strength to root of hair	76
207.	*Vanilla planifolia* (Jacks. ex Andrews)	Flat-leaved Vanilla, Tahitian Vanilla West Indian Vanilla (E)	Fruit	Aphrodisiac and main source of vanilla	35
208.	*Vanilla walkeriae* Wight	-	Stem	It is used to cure fever in cattle and nutritive supplement	93
209.	*Zeuxine strateumatica* (L.) Schltr.	Lawn orchid, Soldier's Orchid (E)	Tuber	Source of tonic and salep.	21

The important medicinal orchids are *Habenaria intermedia* (Riddhi), *Platenthera edgeworthii* (Vridhi), *Malaxis acuminata* (Rishbhak), *Malaxis muscifera* (Jeevak), *Dendrobium macraei* (Jivanti) and *Satyrium nepalense* (Salam misiri) [2]. The first four of the above-listed species are the components of 'Asthavarga' (group of eight medicinal plants) that is a vital part of highly popular polyherbal formulation "Chyvanprasha", widely used as a tonic, rejuvenator, anabolic, immunomodulator and memory enhancer [2,6,50,72]. However, the most important medicinal orchid is *Dactylorhiza hatagirea* (Salam Panja).

In addition to the 'Asthavarga' orchids, a number of orchids are well known for their significant medicinal properties along with their ornamental merit. Antibacterial activities of *Aerides multiflorum* and *Coelogyne nitida* have been reported against *Salmonella auereus* and *Klebsiella pneumonia* [4,22], whereas *Dendrobium farmeria* inhibits the growth of *Escherichia coli, Salmonella typhi* and *Klebsiella pneumonae* [4]. Recently, methanolic extract of *Satyrium nepalense* has been shown to possess excellent antibacterial activities against gram (+)ive and gram (-)ive bacteria, namely *Staphylococcus mutans, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiellla pneumonia*, which are pathogenic for human beings [94]. Distinctive blood clotting attribute of decoction obtained from crushed leaves of *Cymbidium giganteum* was reported by [55]. [17]demonstrated that 6-8 drops of decoction of roots of *Habenaria commelinifolia* administered orally in empty stomach for 10 days cures urinary troubles and spermatorrhoea. *Habenaria edgeworthii* have shown excellent rejuvenating and disease preventing properties [2]. Dried tubers of this species are used to cure skin and cardiovascular diseases. *Dendrobium* has been used as a source of tonic, antipyretic, astringent, aphrodisiac and anti-inflammatory compounds[56, 96]. Antioxidant activity and production of phenolic compounds in *Habenaria edgeworthii*, an important 'Ashtavarga' plant, has been confirmed by [97]. Singh and Tiwari, (2007) demonstrated therapeutic potential of different *Eulophia* species for various ailments like fertility, aphrodisiac, anti-rheumatic, antifutigue, skin protective, wound healing and antitumour activity[98]. The chloroform extract of *Cymbidium aloifolium* showed significant antibacterial activity against ten potential clinically pathogenic bacteria, viz. *Klebsella oxytoca Proteus vulgaris, Pseudomonas aerosinosa, Pseudomonas mirabilis, rah anginosus Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus mitis and Xanthomonas sps.* [99]. Chinsamy *et al.*, (2014)confirmed anti-inflammatory, antioxidant and anti-cholinesterase activity of seven South African medicinal orchids, viz. *Ansellia africana, Bulbophyllum scaberulum Cyrtorchis arcuata, Eulophia hereroensis, Eulophia petersii, Polystachya pubescens* and *Tridactyle tridentata*[100]. Recent investigation of Bhattacharya *et al.*, (2015) on chemical profiling of *in vitro* raised plants of *Dendrobium thyrsiflorum* revealed the presence of different secondary metabolites and significantly higher antioxidant activity of *in vitro* raised shoots than those from the mother plants[101].

The therapeutic properties of orchids are due to the presence of secondary metabolites, such as, flavonoids, alkaloids, glycosides and other phytochemicals [2,102,103]. Bisbenzylerianin, an active principle isolated from *Dendrobium chrysotoxum* is an antioxidant [104], whereas 'habenariol' an active principle isolated from *Habenaria edgeworthii* acts as a repellent against herbivores [105]. The tuberous roots of *Eulophia* species are rich in bioactive substances, Eullophiol, Ephemeranphol, Fimbriol, Lusianthridin, Nudol, β-Sitosterol and β-Sitosterolglucoside [98]. The methanolic extract of *Cymbidium aloifolium* has been reported to contain alkaloids anthraquinones, flavonoids, simple sugars, tannins, terpenoids, etc. [99]

NEED OF TISSUE CULTURE

Since long tissue culture techniques have been used for propagation of rare, endangered and threatened orchids [106]. A single capsule contains millions of microscopic seeds [11-14]. Only about 1% of these are able germinate. If all these had the ability to develop onto plants, entire world would have been full of orchids [107]. Rate of germination in orchids is relatively low due to failure of endosperm development and lack of fungal infection. Besides, a skinny cuticle around a small embryo is not adequate to protect it against desiccation [108].

In vitro methodologies circumvent these difficulties using which almost all seeds can be made to germinate on simple defined media, containing sugar, under controlled temperature and humidity conditions. Sometimes mutualistic association proves fatal for orchids due to the formation of net like structure around embryo and secretion of harmful substances by fungi and algae [109]. To bypass this symbiotic relationship and severe consequences of injurious products, tissue culture is the only method, which provides all the required factors necessary for seed germination and seedling growth [109].

Multiplication by mean of vegetative propagation is extremely slow and time consuming to generate large quantity of orchids replica [110]. Its slow growing properties hardly fulfill the need of people, market and various pharmaceutical companies [13]. Long maturation process even reduces its market value [11]. *In vitro* methodology can reduce the length of time needed for germination and large scale multiplication [110]. Consequently, tissue culture technique has wide range of application in micropropagation of orchids and the only approach to save these critical sources of medicine.

METHODS OF ORCHID MICROPROPAGATION

Several media have been tested for asymbiotic *in vitro* germination of orchid seeds. The most commonly used media are MS [111], Mitra's [112] and Knudson [113]. Other media like p723 (PhytoTechnology Orchid seed sowing Medium),[114] VW (Vacin and Went Modified Orchid Medium)[114], MM (Malmgren Modified Terrestrial Orchid Medium,[114] BM-1(Terrestrial Orchid Medium),[114] HP (Hyponex peptone medium)[115] and NDM (New Dogashima Medium)[116] have also been used for *in vitro* culture of orchids. However, detailed *in vitro* studies are available only for few genera viz., *Acampe, Bletia, Cleisostoma, Cymbidum, Cypripedium, Dactylorhiza, Dendrobium, Epipactis, Eria, Geodorum, Goodyera, Grammaophyllum, Habenaria, Laelia, Malaxis, Oncidium, Paphipedilum, Phalaenopsis, Rhynchostylis, Vanda* and *Vanilla.* These are highlighted in Table 2.

Seed germination

Orchid seeds do not possess enzymes to metabolize polysaccharides and lipids[11]. Besides, being non-endospermic they lack sufficient reserve food material to support growth of embryo. Therefore, to fulfill this requirement, seeds enter into symbiotic relationships with various mycorrhizal basidiomyceteous fungi to provide required nutrients for their germination[14]. Fungal mycelia enter the seed, penetrate the germinating embryo and relationship between fungus and seed is established. Endomycorrizal fungi break down starch to release sugar for utilization by the developing embryo. Symbiotic fungus provides the embryo organic material, water and mineral nutrients[117]. In nature, high proportions of seeds fail to survive and germinate as the mycorrhizal association is not common. To overcome this problem Knudson, (1922) established methodology for asymbiotic seed germination on suitable artificial medium under controlled conditions[109]. He demonstrated that orchid seeds could germinate on simple nutrient medium containing sugars without mutualistic relationship[109]. Knudson, (1930) also highlighted that obligate symbiosis was not necessary either for seed germination or for flowering[118].

Germination potential of embryos varies depends on their developmental stage[119]. The immature seeds germinate readily and much better than the mature ones. This conclusion is based on studies on *in vitro* germination of seeds of *Cypripedium calceolus*, *Dactylorhiza maculata*, *Epipactis helleborine*, *Goodyera repens*, *Gymnadenia conopsea*[120] *Dendrobium florum*, *Cymbidium elagans*[110], *Satyrium nepalense*[121], *Habenaria edgeworthii*[122], *Acampe papillosa*[123], *Dendrobium thyrsiflorum*[101]. On the other hand, some investigators have reported *in vitro* germination of mature seeds of *Phalaenopsis gigantean*[116], *Cymbidium sp.*[125] was better than immature seeds. During asymbiotic germination, embryo swells to from a spherule which develops absorptive epidermal hairs known as rhizoids[126].

The embryo is oval shaped with larger cells at basal region and smaller meristematic cell at upper region, whereas in the later stages of development there is formation of achloropyllous and chlorophyllus protocorm like bodies[109].

The term protocorm was first used by Treub, (1884) during analysis of sporophyte development in lycopodiaceae[127]. Morel (1960) introduced the term protocorm like bodies (PLB) for protocorms developing in orchid tissue cultures[128]. Rasmussen, (2002) Considered protocorm equivalent to radical and hypocotyls of seedlings of other plants[15]. Shape of the protocorm is not constant and these are spherical, oval, round, oboviform, elongated branched, disk, spindle or thorn shaped[129-130]. Protocorm of *Calypso bulbosa* are round[131], whereas those of *Goodyera repen* are elongated[132].

In vitro germination of seeds has been successful in *Geodorum densiflorum*[133], *Cypripedium calceolus*, *Dactylorhiza maculata*, *Epipactis helleborine*, *Goodyera repens*, and *Gymnadenia* *conopsea*[120], *Malaxis khasiana*[12], *Oncidium sp.*[16], *Rhynchostylis retusa*[134], *Dendrobium candidum*[135], *Bletia purpurea*[114], *Satyrium nepalensis*[121], *Laelia speciosa*[136], *Cymbidium elagans, Dendrobium densifolium*[110], *Eria bambusifolia* [13], *Paphiopedilum sp.*[137], *Cymbidium giganteum*[42], *Habenaria edgeworthii*[122], *Cymbidium aloifolium*[138], *Dendrobium aphyllum*[14], *Phalaenopsis gigantean*[116], *Acampe papillosa*[123] and *Dendrobium thyrsiflorum*[101].

Factors affecting seed germination

Non symbiotic seed germination of orchids is greatly influenced by several factors like seed age, different media, PGR, sugar, carbohydrates, vitamins, temperature, light, pH, atmospheric conditions, moisture and orientation of the explants on the medium[109,139]. *In vitro* seed germination of mature seeds is generally a difficult task[108]. Thus, only 13 and 31% of 200 day old seeds of *Paphiopedilum villosum* var. *densissimum* germinated on KC medium after 40 and 80 days of culture, respectively[137]. However, about 70% of mature seeds of *Acampe papillosa* germinated and differentiated protocorms on being cultured on Mitra's medium supplemented with coconut water (15%) [123].

Choice of sugar used in the culture medium too influences germination of seeds and further growth of seedlings[139]. Moreover, requirement of sugar varies with different developmental stages of seed germination[140]. L-glucose and L-mannose failed to support germination of seeds of *Cymbidium elagans* and *Coelogyne puntulate*, while other sugars, such as, sucrose, D-glucose, maltose, trehalose and raffinose significantly enhanced germination frequency of these plants of [137,141], which was in accordance with analysis of [142].

According to Harvais, (1982) cytokinins are the most important growth regulators for germination of ground orchids[143]. Arditti and Ernst, (1984) Opined that orchid seeds are more sensitive to higher cytokinin levels than the protocorm[144]. Deleterious effect of 2,4-D on seed germination and differentiation of protocorm is reported by many authors[141,145,146]. Pradhan and Pant, (2009) Observed better seed germination in *Cymbidium elagans* when BAP (1mg/l) along with NAA (0.5mg/l) was added to the medium[110]. Swar and Pant, (2004)also reported similar results for seed germination of *Cymbidium irridioides*[147]. The frequency of germination of seeds of *C. irridiodes* was the maximum when MS basal medium was used for *in vitro* seed germination of *C. irridoides*[148]. Similar results were obtained by Pant and Gurung, (2005) for *Aerides odorata*[149].

Peptone is a water soluble protein with high contents of amino acids, amides and rich in vitamins which stimulate seed germination [150]. According to Hossain *et al.*, (2010), seed germination and health of protocorm depends on peptone supplemented to the culture media used for germination of seeds of *Cymbidiuim gigantem*[42]. Study made by Curtis, (1947) indicated that very little concentration of peptones (0.05%) proved synergistic to seed germination in *Paphiopedilum* and *Vanda sp*[151]. Recent reports even observed that peptone supplemented Mitra's medium enhanced seed germination in *Herminium lanceum* and *Satyrium nepalense*[152,153]. In contrast, peptone reduced seed germination of *Habenaria clalvellata* [11].

Biotin and nicotinic acid along with pyridoxine promoted seed germination of *Orchis laxisflora* [154]. Sharma *et al.*, (1991) reported that presence of many vitamins in the media favored germination and growth of seedlings of *Cymbidium elagans* and *Coelogyne punctulata*[155]. Depauw *et al.*, (1995) observed synergistic role of BAP with modified barley medium in enhancement of seed germination of *Cypripedium spp*[156].

PROLIFERATION AND DEVELOPMENT OF MULTIPLE SHOOTS

Role of cytokinins has been variously addressed in *in vitro* studies on orchids. TDZ is one of the most commonly used growth regulators in orchid tissue cultures. Mahendran and Bai, (2009) reported maximum frequency of multiple shoots (14.62 shoots/explant) formation in *Satyrium nepalense* on MS medium supplemented with 13.76µM TDZ and it reduced significantly on the same medium containing lower concentration of TDZ (4.52µM) [121]. The effectiveness of TDZ in shoot proliferation has also been reported for *Anoectochilus formosanus* [157], *Dendrobium* hybrids [158], *Dendrobium candidum* [159] and *Phalaenopsis gigantean* [116], *Herminium lanceum* [153].TDZ has been reported to adversely influence elongation and rooting of regenerated shoots of *Pinus strobus*(cited in [160]. This might be due to its greater persistence power to stay inside tissues in contrast to other adenine type cytokinins, BAP or KN [160] . Huang *et al.*, (2001) found deleterious effects of TDZ on proliferation of shoots and rooting of *Paphiopedilum* hybrid[124]. Nayak *et al.*, (1997 a, b) developed an effective protocol for the elongation shoots of *Acampe praemorsa*, *Cymbidium aloifolium*, *Dendrobium moschatum* to circumvent the problem earlier encountered by Huetteman and Preece, (1993) by transferring the shoot clumps regenerated on MS+TDZ to MS +0.5mg/l BAP+2mg/l NAA[160-162].

TDZ was more successful than rest of the cytokinins in inducing multiple shoots from different explants of *Acampe praemorsa* [161,162]. Rao *et al.*, (1993) observed that number of shoots and leaves of *Vanilla planifolia* significantly reduced with increasing concentration of BAP[163]. This is in tune with the findings of Bhatt, (1994), who reported that increasing the concentration of cytokinin proved deleterious for shoot growth of *Vanilla planifolia*[164]. Similar results were obtained by Neelannavar *et al.*, (2011), who observed that lower concentration of BAP (1.5 mg/l) than the higher levels in MS medium resulted in more shoots (4.70 per explant) of *Vanilla planifolia*[165]. High frequency of shoot formation within four weeks of culture of rhizome sections of *Geodorum densiflorum* was observed on MS medium fortified with 5µM BAP [166]. Apart from cytokinins, Tan *et al.*, (2013) evaluated the effect of sodium nitroprusside on shoot regeneration and multiplication of *Vanilla planifolia*, where the number of shoots/explant showed a significant increase in the presence of SNP and more than 93% of the explants formed shoots[167]. In *Malaxis acuminata*, about 98 percent of pseudobulb segments responded positively and formed 11 shoot buds/explant within 5-6 week of culture on MS medium enriched with CH (100 mg/ L), NAA and BAP (6 µM each) [168]. In *Dendrobium thyrsiflorum,* the maximum (96%) regeneration frequency along with an average 17.7 shoots/explants with a mean length of 3.5 cm was observed on MS medium containing 2 mg/l TDZ and 0.5 mg/l NAA [101].

Among different auxin-cytokinin combinations, BAP (0.2, 5 mg/l) and NAA (0.1, 0.5 mg/l) promoted shoot organogenesis in four species of *Paphiopedilum*, *P.densissimum*, *P. insigne*, *P. bellatulum*, and *P. armeniacum* [137]. Likewise, regeneration of plantlets and PLB proliferation in *Malaxis khasiana* was better on MS medium supplemented with NAA (10 µM) and BAP (8µM) than on MS basal medium [12]. Similar results were obtained in *Grammatophyllum speciasum*[169], *Oncidium sp.* [16], *Rhynchostylis retusa* [134] and *Geodrum densiflorum* [133].

Contrary to above reports, Dutta *et al.,* (2011) reported that auxin-cytokinin interaction was not beneficial for the proliferation of PLBs into multiple shoot production in *Dendrobium aphyllum*[14]. It was possible to induce multiple shoot formation if IAA or KN were used individually. In *Malaxis acuminata,* all responding explants produced single adventitious shoot irrespective of the type and concentration of the cytokinin, but addition of 0.5mg/l NAA to the medium enhanced adventitious shoot formation [170].

PLANT REGENERATION IN CALLUS CULTURES

Callus cultures of orchids have shown limited success because of difficulty in callus induction, their limited growth often accompanied with severe necrosis [55,106,171,172]. A number of authors feel that initiation and subculture of callus in orchids is challeng [171,172,173,174,175]. Nevertheless, several investigators have reported beneficiary role of exogenous auxins in callus production, maintenance and development in a number of orchids,e.g. *Cymbidium ensifolium* [176], *Paphiopedilum* hybrid [104], *Dendrobium fimbriatum* [177], *Cymbidium sp.* [178,179] *Pahiopedilum sp.* [180]. [181] made an effort for long term callus cultures of *Paphiopedilum*. Induction of totipotent calli from seed derived protocorms of *Cypripedium formosanum* (slipper orchid) on 1/2 MS+2,4-D(0-5mg/l)+TDZ(1mg/l) was reported by Lee and Lee, (2003) and Lu, (2004) [182-183]. Friable and light yellow callus was induced within eight weeks, when seeds of *Habenaria edgeworthii* were cultured on MS medium or same fortified with 1 µM NAA. This was sub-cultured repeatedly after four-week intervals to increase its biomass [97].

Somatic embryogenesis is not well documented for orchids [175,184]. However, Chen *et al.,* (1999) observed the development of somatic embryos on leaf tip explants of *Oncidium*[61]. Roy and Banerjee, (2003) and Roy *et al.,* (2007) observed the formation of embryogenic callus from shoot tip explants of *Dendrobium fimbriatum* and *Denrobium chrysotoxum*[55-177].

ROOTING, HARDENING AND ACCLIMATIZATION OF PLANTLETS

Rooting of *in vitro* regenerated shoots is a critical step in any micropropagation protocol. The roots developed should be hardy enough to support the plantlets on being transferred from *in vitro* conditions to ambient ones. Generally, an auxin or rarely a combination of auxins is used for the rooting of shoots of orchids. Hossain *et al.,* (2010) reported development of solid root system from PLBs and shoot buds of *Cymbidium giganteum* inoculated on half strength of Phytamax or Mitra's medium supplemented with 0.5mg/l IAA. In *Vanilla planifolia*, IBA alone at 0.5mg/l proved to be the best in inducing the highest number of roots along with good length in small time [42-165]. The efficiency of IBA in root induction has also been observed in *Cymbidium pendulum* [185]. These results are also consistent with the findings of Mohanty *et al.,* (2012), who successfully rooted regenerated shoots of *Dendrobium nobile* by transferring them to MS medium containing 1.5 mg/l IBA[186]. Likewise, *in vitro* shoots of *Dendrobium thyrsiflorum* rooted best on 1/2 MS medium supplemented with 1 mg/l IBA and 0.5 mg/l phloroglucinol (Bhattacharya *et al.,* 2015) Even the *in vitro* raised shoots of *Satyrium nepalense* were rooted on MS medium fortified with 9.84µM IBA [101-121] .Similarly, in *Hermnium lanceum* best rhizogenic response was observed in 0.1 µM IBA supplemented Mitra's medium [153]. However, IAA and IBA were not always effective in inducing roots in many species of orchids. Sheelavanthmath *et al.,* (2000) reported ineffectiveness of IAA and IBA in induction of roots from shoots of *Geodorum densiflorum* , 100% of which developed shoots on medium containing NAA (1µM) [166]. The combination of NAA with BAP proved to be differentiation of shoots and their rooting in *Grammatophyllum speciasum*, *Oncidium sp.*, *Thynchostylis retusa* and *Geodrum densiflorum* [16,133,134,169].

Werckmeister, (1971) first used charcoal to darken the medium for culture of shoot tip derived *Cymbidium* plantlets[187]. This was followed by Ernst, (1974 (a,b) 1975) who used it for seed germination of *Paphiopedilum* and *Phalaenopsis*[188,189,190]. Cheruvathur *et al.,* (2010) observed that the presence of activated charcoal was compulsory for root induction in *Malaxis acuminata*, irrespective of the auxin used[170]. This could have been due to reduction of light at the base of plants because of the inclusion of charcoal in the medium, thus resulting in reduction of inactivation of photosensitive auxin (IAA) absorption of inhibitory substance, such as, polyphenols (Pan and Staden, 1998), adsorption of high concentration of growth regulator like IAA, NAA, IBA, BA, KN (Weatherhead *et al.,* 1979) and ethylene [191-193]. Eymar *et al.,* (2000) observed that AC maintains pH, increases nitrogen uptake, improves growth and reduces inhibitory effect of exogenous cytokinin on rooting[194]. Piri *et al.,* (2013) too reported formation of root primodia in *Acampe papillosa* when Mitra's medium was fortified with AC (2g/l), CW (15%) and YE (2g/l) [123].The incorporation of lower concentration of activated charcoal (< 0.3%) in the MS medium promoted healthy root formation and pigmentation of the plantlets in

Malaxis acuminate [168]. Critical role of AC in root induction has also been reported in *Rananthera imschootiana* [195]; *Anoectochilus formosanus* [157] *Cymbidium faberi* [196] and *Dendrobium hybrid* [158]. Gruenschneder, (1973) reported that AC reduced browning and stimulated root development in *Dactylorhiza maculate*[197]. In *Cymbidium*, it assisted in establishing polarity so that roots become positively geotropic [187].

Effective and successful tissue culture can only be realized when plantlets are transferred from *in vitro* to ambient conditions [198]. A wide variety of potting mixes (substrata) have been used for the transfer and acclimatization of in vitro raised orchid plantlets. Giri *et al.*, (2011) reported maximum (87.5%) rooting when elongated shoots were transferred to half strength MS basal medium, where shoots developed tuberous roots after two months of culture[122]. Nearly 68% survival rate was recorded when shoots of *Habenaria edgeworthii* with elongated roots were transferred to a mixture of soil:sand:perlite (1:1:1) ratio. Similar results were also obtained for *Habenaria bractescens* (Medina *et al.*, 2009) and *Habenaria macroceratitis* [199-200] . Franco *et al.*, (2007) appraised the effect of ten substrates (pine bark, coco fibre, wood shaving, polystyrene foam etc.) on establishment of *in vitro* raised plants of *Cattleya trianae*[201]. On potting mixes comprising pine:coco:fibre,coal (1:1:1), coco:fibre (1:1) and pine:coco fibre (1:1) 60, 76 and 86%, respectively of the transferred plants survived. The lowest survival (12%) was on pine bark. Rooted shoots of *Malaxis acuminata* survived well when transferred to a potting mixture of charcoal chips and soil (1:1), covered with polybags and mist irrigated with half strength of MS liquid media [170]. The maximum survival (82%) of rooted shoots of *Dendrobium nobile* was obtained in the compost consisting of charcoal chunks and brick pieces (1:1) with a top layer of moss with [167]. Tan *et al.*, (2013) reported 85.0% survival rate after 4 weeks of acclimatization, when *in vitro* well developed rooted shoots of *Vanilla planifolia* were transferred to potting mixture having sand and compost (1:2) [186]. However, when substratum containing chopped forest litter, coco pits and sand (1:1:1) was used for acclimatization of *Malaxis acuminata*, 75% survival was observed after 2 month of transfer [168]. Recently, *in vitro* rooted shoot of *Satyrium nepalense* and *Herminium lanceum* were successfully hardened in pots having potting mixture of sand and vermiculite(1:1)[152-153].

A summary of some of the *in vitro* studies on orchids is provided in Table 2.

Table 2-Some recent tissue culture studies on orchids.

Taxa	Type of Culture	Medium Used	pH Adjusted	PGR (mg/l)	Other Supplements	Remarks	Investigators
Acampe papillosa (Lindl.) Lindl.	Seed	M, PDA	-	-	AC(2g/l) CW (15%) YE(2g/l)	M+CW = maximum (70.75%) germination	123
Aerides crispum Lindl.	PLB, leaf	MS	5.6	BAP (0.1 to 1.12), N (0.1 to 1), TDZ (0.1 to 1.1), AA (0.08 to 0.8), AA (0.09 to 0.9)	Sucrose (2%) Agar (1%)	BAP at 5.0 μM induced multiple shoots	202
Bletia purpurea(Lam.) DC.	Seed	KC, ½ MS, BM-1, MM, VW, P723	5.8	-	Sucrose (2%)	Photoperiod stimulated seed germination	114
Cleisostoma racemiferum(Lindl.) Garay	Seed	MS,M, KC	5.6	NAA (0 to 5.6), BA (0 to 0.2), IAA (0 to 3.5), KN (0 to 3.9),	Sucrose (3%) Agar (0.8%)	MS+IAA (1.7 mg/l) + BA (1.8 mg/l) = multiple plantlet MS+NAA (1.8 mg/l) + KN (1.9 mg/l) = well differentiated root	12
Cymbidium aloifolium (L.)	Seed	MS,M, KC	5.6	BA,TDZ,KN (0 to 2), IAA, NAA (0 to 1.6)	Sucrose (2%) Agar (0.8%)	MS+NAA(0.5 mg/l)+BA(0.1 mg/l) = 90% seed germination	138
Cymbidium elegans Lindl, .*Dendrobium densiflorum* Lindl. ex Wall.	Seed	MS	5.8	BAP (0.5 to 2), NAA (0.5)	Sucrose (3%) Agar (0.8%)	MS+BAP (1 mg/l) = Stimulated seed germination	110

Taxa	Type of Culture	Medium Used	pH Adjusted	PGR (mg/l)	Other Supplements	Remarks	Investigators
Cymbidium, Epidendrum, Oncidium, Paphiopedilum and Phalaenopsis.	Pseudo bulb, rhizomes, Roots	MS, ½ MS	-	TDZ (0.1 to 1), 2,4-D (1 to 10), NAA (0.1 to 0.5), BAP (5)	Sucrose (4%)	Different concentration of NAA and TDZ formed embryos and maintained platelets development	203
Cymbidium giganteum Wall. ex Lindl	Seed	MS, KC, PM, M	5.8	BAP (0 to 2), IAA, 2,4-D (till 2)	Sucrose Agar (0.8%) AC (2%) Peptone (2g/l)	M/PM+peptone(2g/l)+BAP(1mg/l) = 100% seed germination M/PM+AC = largest PLB	42
Cypripedium macranthos var. rebunense	PLB	¼ MS, HP	5.5	NAA (till 0.5), BAP(till 0.22), Zeatin(2.2)	Sucrose (2%) Agar (0.6%)	HP with NAA and cytokinin proved best for PLB proliferation	115
Dendrobium aphyllum (Roxb.)	Seeds	MS	5.8	IAA (0 to 0.5), KN (0 to 0.5)	-	IAA(0.5 mg/l) = maximum shoot length	14
*Dendrobium candidum*Wall. ex Lindl.	Seed	MS, ½ MS	5.8	KN (0 to 2.9), BAP (0 to 5), NAA (0 to 1), 2,4-D (0 to 3)	Sucrose (2%) Agar (0.6%)	MS+BAP(1.98mg/l) = highest callus induction	135
Dendrobium nobile Lindl.	Shoot tip	M	5.8	TRIA (1 to 5 mcirogram/l)	Sucrose (3%) Agar (0.8%)	Effective range of TRIA is 2-7 µg/l	204
Dendrobium thyrsiflorum Rchb.f	Seed, nodal segment	MS	5.8	BAP, KN, TDZ (each 0 to 4mg/l)+ NAA(0.5mg/	Agarose (0.8%)	MS+TDZ(2mg/l)+NAA(0.5mg/l) = maximum 17.7 shoots proliferated	101

Taxa	Type of Culture	Medium Used	pH Adjusted	PGR (mg/l)	Other Supplements	Remarks	Investigators
				l)			
Eria bambusifolia Lindl.	Seed	MS, KC	5-5.8	NAA, BA, KN, GA3 (0.5,1,2)	-	MS+IAA(2 mg/l) = enhanced shoot length, MS+NAA(2 mg/l) = Best rooting	13
Geodorum densiflorum (Lam.) Schltr.	Seed	MS, ½ MS	5.4-5.8	NAA (till 2), BAP (1 to 2.5), IAA (1), Zeatin(1),	Sucrose (3%) Agar (0.8%)	NAA(2 mg/l)+BAP(2 mg/l) = enhance elongation IAA(1 mg/l) = root system developed	133
Grammatophyllum speciosum Blume	PLB	MS, ½ MS	5.7	NAA, BAP (0 to 2)	Sucrose (2%)	MS+NAA(2mg/l)+BAP (1mg/l) = Optimum shoot formation	169
Habenaria bractescens Lindl.	Multi modal stem	MS	5.5	BAP (1 to 10)	Sucrose 87.6 milli molar Agar (0.65%)	BAP at 10mg/l stimulated root tuber formation	199
Habenaria edgeworthii Hook.f. ex Collett		MS, ½ MS	5.6	NAA (0 to.09) BAP, IBA (0 to 0.1)	Agar (0.8%)	NAA (1μM) = max seed germination' BA+NAA = maximum shoot	122
Habenaria radiata(Thunb.) K. Spreng	Shoot apex and leaf	½ MS	5.6	BAP, NAA	Sucrose (3%) Agar (0.8%)	1/2MS+BAP(0.54 μM+NAA(4.44 μM) = highest (5.4) adventitious bud/ floret	205
Laeliaspeciosa(HBK) Schltr.	Seed	KC, MS ,1/2 MS	-	BAP (0 to 0.5), GA3 (till 10), NAA(0 to 1)	Sucrose (3%)	MS+NAA(0.5 mg/l)+GA3(0.1 mg/l) = effective for germination	136
Malaxis acuminata D. Don	Pseudobulb	M	5.7	BAP(1), NAA(1)	Sucrose (2%) Agar (0.9%)	BAP+NAA(1mg/l each) promoted PLB proliferation and plantlet development	206
Malaxis acuminata	Internode	MS	5.8	BAP, KN, TDZ (each 1	Sucrose (3%)	NAA(0.5mg/l) = Enhanced adventitious	170

Taxa	Type of Culture	Medium Used	pH Adjusted	PGR (mg/l)	Other Supplements	Remarks	Investigators
D.Don				to 4) , NAA (0.5)	Agar (8%)	shoot, TDZ(3mgl/l) = 96% organogenesis	
Malaxis acuminata D. Don	Pseudobulb	MS	5.6	NAA, BAP (0 to 9 µM)	Sucrose (3%) CH (100mg/l) Citric acid (100mg/l) AC(0-0.4%)	CH+NAA+BAP (6 µMeach) = induced 11shoot bud/explant after 6week	168
*Malaxis khasiana*Soland ex. Swartz	Seed	MS, M	5.6	IAA (0 to 1), BAP (0 to .07), KN (0 to 5.8), NAA (till 0.5)	Sucrose (2%) Agar (0.7%) AC (0.1%)	MS+IAA(1 mg/l)+BA(4 mg/l)+KN = induced multiple shoot	12
Oncidium sp.	Seed	MS	5.6	BAP, NAA (0 to 4)	Agar (0.8%)	BAP(2)+NAA(1.5 mg/l) = 100% shoot and root forming capacity	16
Paphiopedilum species	Seed	MS	5.8	BAP(1 to 8), KN (0.1), NAA (0 to 1), TDZ (till 0.5) 2,4-D (1 to 8), IAA, IBA	Sucrose (2%) Agar (0.6%)	NAA (0.5,0.1), BAP (4, 5.5 mg/l) = maximum shoot were observed	137
Phalaenopsis gigantea	PLB, leaf, ripe capsule seed	MS, NDM	-	BAP, KN, NAA, TDZ (0 to 1)	Sucrose (2%)	TDZ with NAA was found to be best for PLB and callus induction	116
Rhynchostylis retusa Blume	Seeds	MS	5.8	BAP (0 to 1.8), NAA (0 to 0.8),	Sucrose (3%) Agar (0.8%) AC (0.1%)	BA(1.3 mg/l)+NAA(0.03 mg/l) = Seedling growth maximum TDZ(0.44 mg/l) =	134

Taxa	Type of Culture	Medium Used	pH Adjusted	PGR (mg/l)	Other Supplements	Remarks	Investigators
				IBA (0 to 1.2), TDZ (0 to 1.3), KN (0 to 1.3)		multiple shoot	
Satyrium nepalense D. Don	Seeds	MS,KC, KCM	5.6-5.8	IBA, BAP, KN (each 1 to 4) TDZ (0.5)	Sucrose (3%) Agar (0.8 %)	TDZ(13.76 μM) = Multiple shoot, IBA(9.84 μM)= best rooting	121
Vanilla planifolia Andrews	Node	MS	-	BAP (0.1 to 3), NAA (0 to 1), KN (0 to 1.5)	Sucrose (3%) Agar (0.8%)	BAP(1 mg/l)+KN(1 mg/l) = maximum shoots were obtained after 45 days	207
Vanilla planifolia Andr.	Shoot tip, internodes, leaf segment, bud , root	MS	-	BAP (0.5 to 3)	-	BAP(1 mg/l) = enlarged shoot BAP(0.5 mg/l) = enlarged root	165
Vanilla planifolia Andrews	Node	MS	5.8	SNP (0to 40µM)	Agargel (0.55%)	10 µM SNP+ BAP(1 mg/l) = highest number of shoots	167
Vanda testacea (Lindl.) Reichb. f.	Leaves	M	5.7	KN, NAA, BAP (1)	Sucrose (2%)	BAP(1 mg/l)+NAA(1 mg/l) = PLB proliferation	206

AC: Activated charcoal, BAP: 6-Benzylaminopurine, CH: Casein hydrosylate, 2, 4-D: 2, 4-Dichlorophenoxyacetic acid, GA_3: Gibberellic acid, IAA: Indole- 3-acetic acid, IBA: Indole-3-butyric acid, KC: Knudson C medium (Knudson C 1946), KN: Kinetin, M: Mitra medium (Mitra et al 1976), MM: Malmgren Modified Terrestrial Orchid Medium, MS: Murashige & Skoog,s medium (Murashige & Skoog 1962), NAA: α-Naphthalene acetic acid, PGRs: Plant growth regulators, PLBs: Protocorm like bodies, SA: Syringic acid, SNP: Sodium nitroprusside, TDZ:-1-Phenyl-3-(1,2,3-thiadiazol-5-yl)-urea , VW: Vacin & Went Modified Orchid Medium

SYMBIOTIC SEED GERMINATION

The presence of fungi in orchid roots under natural conditions was first observed by Reissek, in 1847) [208]. Later, in 1866, Wahrlich identified and described various changes taking place in orchids roots due to fungal infection[209]. Magnus, (1900) was the first to observe peloton inside the cells and even described various stages in fungal colonization[210]. Recent report by Singh *et al.,* (2017) even confirmed the presence of pelotons in cortical cells of root section of *Herminium lanceum* and *Satyrium nepalense*[211]. Seeds of orchid are shed when embryos are at few celled stage and rest of its development takes place during germination [212]. Orchid seeds are extremely small and lack sufficient reserve food material to support the growth of embryo. It depends on mycorrhizal association for the nutrition required by the immature embryo to develop into a protocorm. In most of the species, symbiotic association is established by infecting suspensor cell at the base of embryo, whereas in few cases infection occurs through rhizoids [213]. The embryo or protocorm attract symbiotic fungi by producing chemotrophic substances [214,215]. The fungal associates convert insoluble carbohydrates to simple soluble form and thereby, provide organic carbon to the developing embryos [216,217]. The mycorrhizal fungi are also known to supply nitrogenous compounds(Cameron *et al.*, 2006; Burgeff ,1936; Dijk, 1990), soluble phosphate (Smith, 1967; Alexander *et al.,* 1984) and vitamins (Hijner and Arditti, 1973) during seed germination[218-223]. There are many studies reporting increase in percentage of seed germination and protocorm development if seeds were cultured along with fungal isolates. Clements *et al.,* (1986) reported that fungal isolates *Tulasnella sp.* and *Ceratobasidium* stimulated seed germination in *Orchis sp.* and *Dactylorhiza elata,* respectively[224]. Generally, during symbiotic seed germination continuous exposure in dark is required [225]. However, Zettler and Mclnnis, (1994) noticed a synergistic effect of 16 hr photoperiod, during the first 7 days of inoculation, in endangered terrestrial orchid *Platanthera integrilabia*[226]. Zettler, (1997) applied symbiotic seed germination technique for the conservation of terrestrial orchids, *Platanthera* spp. (*P. cristata, P. integrilabia, P. clavellata*), *Spiranthes odorated*[227]. In the presence of fungal associates 3 % seeds of these taxa germinated within two weeks. Stewart and Zettler, (2002) observed that the percentage of seed germination in *Habenaria quinqueseta, Habenaria macroceratitis* and *Habenaria repens* increased to 18.1, 50.8 and 55.1%, respectively from less than 1% in controls, after incubation with *Ceratorhiza* isolates[228]. Athipunyakom *et al.,* (2004) isolated *Epulorhiza repens* and *Rhizoctonia globularis* form the roots of *Spathoglottis plicata*[229]. In the cultures of the seeds of the same plant inoculated with these fungi, the percentage of seed germination recorded after 127 day of culture were 42.8% (*E. repens*) and 12.5% (*R. globularis*), as opposed to the total absence of germination in control. There is an obscure liaison between orchids and endophytic fungi that whether the fungi isolated form adults plant are also crucial for their seed germination [225]. Chutima *et al.,* (2011) evaluated effect of endophytic fungi on seed germination of *Pecteilis susannae*. Seed germination enhanced significantly from 62.1% in controls to 79.9% when cultured along with fungal isolates, *Epulorhiza sp*[230].

CONCLUSIONS

Present review has made an effort to bring together all possible literature of *in vitro* propagation of orchids via seeds, rhizomes, shoot tips, internodes, pseudobulbs, PLBs, leaves, roots, node as explants (Table 2). Orchids are rich in demand especially in the field of horticulture due to their splendid glamorous long lasting flower. The protocols already developed can possibly be used for large scale mass multiplication along with *ex vitro* establishment of rare, threatened and endangered orchids to meet the horticultural and floricultural market demand. Cost effective protocol by using minimal media and inexpensive substitutes such as gelling agent, sugar source and concentration, vitamins etc. need to be developed to facilitate commercialization and conservation programs. Taking threat into consideration, orchids have been placed in Appedix II and some have been included in even Appendix I of Convention on International Trade in Endangered Species of Flora and Fauna (CITES). Therefore it is the most imperative responsibility of human being to save these critical sources of medicine for human welfare.

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