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In vitro flowering - A review

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In vitro flowering has become a valuable tool assisting micropropagators to release new species and cultivars into market more rapidly. In this review the progress made in the industry of cut flowers, pot flowers and the potentiality for future expansion of this field are highlighted. Flowering involves the conversion of the apical meristematic initials into a floral meristem, from which all the parts of the flower will be produced. Signals that change the fate of the apical meristem include maturity of the plant, temperature, photoperiod and the relative length of day and night. The authors also induced *in vitro* flowering in orchid and some of the endangered and endemic medicinal plants. The present review highlights about 40 families, 75 genera and more than 100 species for the last seven decades.

Key words: *In vitro* flowering, Plant tissue culture, Plant growth regulators.

Introduction

The plant flowers only when genetic factors, including photoperiod, environmental responses are hospitable (Tissarat and Galletta, 1995). These conditions can often be altered, so that the plant can be induced to undergo an early reproductive phase. An investigation report after *Ribes nigrum* reveals that the juvenile like condition has negative effect on the *in vitro* flowering (Schwabe and Al-Doori, 1973). Such an attempt to induce flowering *in vitro* from juvenile explants of some plants is reported here. *In vitro* flowering also facilitates the understanding of physiology of flowering and largely depends upon the level and interaction of exo and endogenous phytohormones, sugars, minerals and phenolics. Induction of floral stimulus, translocating them and floral morphogenesis are the key steps involved here. Several reports have demonstrated that there is considerable variability in the requirements of plant

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growth regulators, temperature, light regime and nutritional factors for *in vitro* flower development in explants from different species (Bernier *et al*., 1981) and experimental evidence supports a multiplicity of factors regulating the *in vitro* flowering process.

In vitro flowering bears immense importance in selective hybridization especially in using pollen from rare stocks and may be the first step towards the possibility of recombining genetic material via *in vitro* fertilization in otherwise non hybridizable lines. Scorza (1982) reviewed *in vitro* flowering for the first time, after which the researchers concentrated *in vitro* flowering of various species. Ramanayake (2006) reviewed on *in vitro* flowering of bamboo species. Conventional orchid breeding is a lengthy process, due to long juvenile phase of orchids, the entire breeding cycle could be of 3-5 years depending on the genotypes involved (Hee *et al.,* 2009). Many workers succeeded in the induction of *in vitro* flowering in juvenile *Dendrobium* hybrids (Sim *et al*., 2007; Tee *et al*., 2008; Hee *et al*., 2007). The role of growth regulators in flowering as demonstrated by *in vitro* techniques was sketched by Nitsch (1972). Demeulemeester and De Proft (1999) studied the *in vivo* and *in vitro* flowering response of chicory. van Staden and Dickens (1991) induced *in vitro* flowering and studied its relevance to micropropagation. An *in vitro* flowering mechanism is considered to be a convenient tool to study specific aspects of flowering and whole mechanisms of the reproductive process such as floral initiation, floral organ development and floral senescence. There are relatively fewer reports on monocot than dicot flowers and inflorescences cultured *in vitro* (Table 1). Explants of different stages cultured and the requirement of plant hormones and nutrients for flower development was also variable. Therefore it is very difficult to present a comprehensive picture on the *in vitro* flowering process. So, the present review mainly focused on the role of various factors and developmental strategies *in vitro*. Morphogenesis of flower is an area in its own right and is out of the scope of this review. The authors were able to induce *in vitro* flowering in endangered and endemic ornamental, medicinal and food plants like *Spathoglottis plicata* (Murthy *et al*., 2006) (Fig-1A), *Ceropegia spiralis* (Murthy *et al*., 2010) (Fig-1 C), *Ceropegia pusilla* (Murthy and Kondamudi 2010)(Fig-1D), *Brassica nigra* (Fig-1B) and *Asparagus recemosus* (Fig-1E), while studying the micropropagation of these species. To induce *in vitro* flowering in the above mentioned species, various concentrations and combinations of plant growth regulators were used. With this background, authors reviewed available literature on *in vitro* flowering. The plants were classified into five groups on the basis of importance of that particular species like ornamental plants, commercial crops, medicinal plants, food crops and endangered / rare / threatened plants for the convenience.

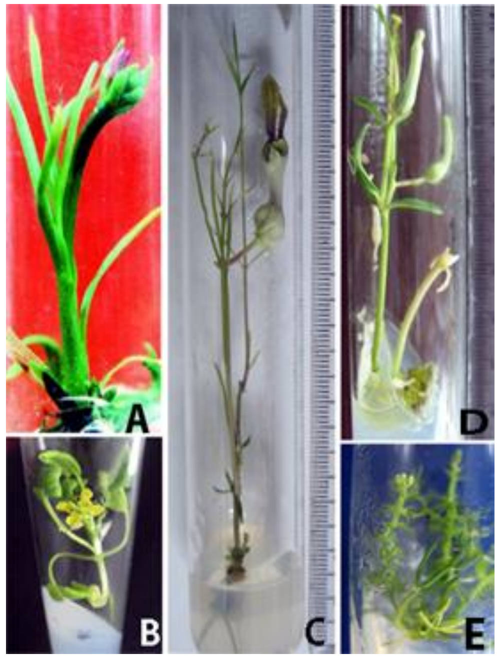

Fig. 1. *In vitro* flowering , A. *Spathoglottis plicata*, B. *Brassica nigra*, C. *Ceropegia spiralis*, D. *Ceropegia pusilla*, E. *Asparagus recemosus*

Table 1. The effect of plant growth regulators on *in vitro* flowering

Species	Sources of explant	Hormones	References
Ammi majus L.	Node	IAA, Kn, CH, Ad, Glu, IBA	Pande *et al.*, 2002
Arabidopsis thaliana(L.) Heynh.	Callus	Ascorbate peroxidase	Lokhande *et al*., 2003
Arachis hypogaea L.	Seedlings	Kn, sucrose	Asawaphan *et al.*, 2005
Arachis hypogaea L.	Mature embryos, leaflets	NAA, BAP, Kn, 2,4-D	Chengalrayan *et al.*, 1995
Arachis hypogaea L.	Seedlings	Kn, sucrose	Narasimhulu and Reddy, 1984
Artemisia annua L.	Vegetative parts	Myo inositol, NAA, BAP, GA_3, Asp, Glu,	Anamika *et al.*, 1996
Bambusa arundinacea (Retz.) Willd.	Nodes, apices	CM, BAP,	Nadgauda *et al.*, 1997
Bambusa arundinacea (Retz.) Willd.	Nodes, apices	AdS, 2iP, Kn, Zeatin, BAP	Mohini and Nadgauda, 1997
Bambusa arundinacea (Retz.) Willd.	Seedling	BAP, 2iP, Kn, Zeatin, AdS	Joshi and Nadgauda, 1997
Bambusa edulis (Odash.) Keng.	Axillary shoots	TDZ, GA_3, ABA, ACC, NAA	Lin *et al*., 2004
Bambusa edulis (Odash.) Keng.	Somatic embryos	TDZ	Lin *et al*., 2003
Basilicum polystachyon (L.) Moench	Shoot tip	BAP, Kn, IBA, IAA	Amutha *et al*., 2008
Boerhavia diffusa L.	Leaf explant	BAP, NAA, Kn, IAA	Sudarshana *et al*., 2008
Boronia megastigma Nees.	Flower heads	Cytokinins, auxins	Robertis *et al.*, 1993
Brassica campestris (L.) var. *bhavani*	Cotyledonary node, shoot apex	BAP, IAA, Kn, IBA, NAA	Verma and Singh,2007

Calamus thwaitesii Becc.	Immature embryos, rhizome, shoots, buds	ACH, NAA, BAP	Ramanayake, 1999
Capsicum annuum L. Cv. Sweet Banana	Immature zygotic embryo	NAA, Silverthiosulphate	Bodhipadma and Leung, 2003
Capsicum frutescens L.	Shoot tips	BAP, NAA	Tisserat and Galletta, 1995
Ceropegia bulbosa var. *bulbosa*	Nodes	GA_3, BAP	Britto *et al.*, 2003
Ceropegia bulbosa var. *bulbosa*	Axillary/apical bud	BAP, Sucrose	Nair *et al.*, 2007
Ceropegia hirsuta Wight & Arn.	Axillary/apical bud	BAP, Sucrose	Nair *et al.*, 2007
Ceropegia jainii Ansari & B.G.Kulk.	Nodes	BAP, Spermine	Patil, 1998
Ceropegia lawii Hook. f	Axillary/apical bud	BAP, Sucrose	Nair *et al.*, 2007
Ceropegia maccannii Ansari	Axillary/apical bud	BAP, Sucrose	Nair *et al.*, 2007
Ceropegia oculata Hook.	Axillary/apical bud	BAP, Sucrose	Nair *et al.*, 2007
Ceropegia sahyadrica Ansari & B.G.Kulk.	Axillary/apical bud	BAP, Sucrose	Nair *et al.*, 2007
Ceropegia spiralis Wight	Nodes	BAP, 2,4-D, IAA, IBA, NAA, Kn	Murthy *et al.*, 2010
Ceropegia pusilla Wight & Arn.	Nodes,	BAP, 2,4-D, IAA, IBA, NAA, Kn	Murthy and Kondamudi, 2010
Chenopodium murale L.	Nodes	BAP, IAA, GA_3	Mitrovic *et al*., 2000
Cichorium intybus L.	TCLs	NAA, BAP, IAA	Harada, 1966
Cichorium intybus L.	Callus	2iP, GA_3, $AgNO_3$, DFMA, DFMO	Bais *et al*., 2000
Cichorium intybus L. cv. *focus*	Leaves	BAP, Kn, Adenine sulphate, IAA, IBA, NAA	Nandagopal and Ranjitha kumari, 2006
Citrus nobilis Lour x *C. delicoisa* Tenora(Kinnow mandarin)	Ovules	Kn, Sucrose	Singh *et al.*, 2006
Citrus unshiu Marc.	Buds and leaves	Cation and anionic solutions	Monerri and Guardiola, 2001
Citrus unshiu Marc.	Buds	Cytokinins	Luis *et al*., 1989
Crocus sativus (Saffron)	Floral buds	NAA, BAP	Jun *et al.*, 2007
Cuscuta japonica Choisy	Stems,	Kn	Furuhashi, 1991
Cymbidium goeringii Rchb.f.	Rhizome	NAA, Banana juice, BAP, GA_3	Ho *et al*., 2003
Cymbidium niveo-marginatum Mak.	Seeds	BAP, 2,3,5 tri iodo benzoic acid, NAA,GA_3, Paclobutrazole	Kostenyuk *et al*., 1999
Dendrobium candidum Wall ex. Lindl	Seeds, Protocorms,	NAA, BA, ABA	Wang *et al.,* 1993
Dendrobium candidum Wall ex. Lindl	Protocorms, shoots, plantlets	Spermidine, BAP, NAA, ABA	Wang *et al*., 1997
Dendrobium Chao praya smile	Seedlings	BAP	Hee *et al*.,2007
Dendrobium Madame Thong-In.	Shoot tips	Modified KC medium, CW, BAP	Sim *et al*., 2007
Dendrobium nobile Lindl.	Seeds	TDZ, NAA,	Wang *et al*., 2009
Dendrobium Second Love	Apical meristem	TDZ, IAA, Zeatin	Ferreira *et al.*, 2006.
Dendrocalamus giganteus Wall. ex Munro	Axillary shoot, nodes	BA, Sucrose	Ramanayake *et al.*, 2001a
Dendrocalamus hamiltonii Munro	Nodes	BAP	Chambers *et al*., 1991
Dendrocalamus latiflorus Munro	inflorescences	2,4-D, PVP, TDZ, NAA,	Lin *et al*., 2006
Dendrocalamus strictus Nees.	Seeds	TDZ	Singh *et al.*, 2000
Drosera burmanii Vahl.	Axillary buds, shoot tips	Kn, BAP	Jayaram *et al.*, 2008
Fagopyrum esculentum Moench	Shoot segments	Kn, high C/N ratio, NAA, GA_3, BAP, IBA	Kachonpadungkitti *etal.,* 2001

Fortunella hindsii (Champ. ex Benth.) Swingle	Node, internodes	BAP, Kn	Jumin and Nito, 1996 b
Fragaria ananassa Duchesne	Shoot apex	BAP	Asao *et al.*, 1997
Gardenia jasmonoides Ellis cv.'Veitchii'	Nodes	Paclobutrazole	de Baerdemaeker *etal.*, 1994.
Gentiana scabra	Leaves, roots, somatic embryos	2,4-D, NAA, BAP	Young-Sook *et al.*, 2005
Kalanchoe blossfeldiana Poellniz	Nodes	Basal medium	Dickens and van Staden, 1988 1990
Kniphofia leucocephala	Shoots	BAP, 2iP, Zeatin, GA_3	Taylor *et al*., 2005
Lemna gibba L.	Seeds	Tobacco smoke and other constituents	Bhalla and Sabharval, 1970.
Lemna minor L.	Axenical cultures	Jasmonic acid, EDDHA,	Krajncic *et al*., 2006
Lemna paucicostata Hegelm.	Seedling	Salicylic acid, aspirin	Khurana and Maheswari, 1978.
Leptinella nana L.	Shoots	MS Medium	Carson and Leung, 1994a
Lilium rubellum Baker	Scales	BAP	Ishimori *et al.*, 2009
Lolium temulentum L.	Seeds	GA_5, GA_6	King *et al.*, 2003 McDaniel and Hartnett, 1996
Lycopersicon esculentum Mill.	Leaf explants	BAP, IAA, IBA	Rao *et al.*, 2005
*Lycopersicon esculentum*Mill.	Leaf, stem	BAP, ABA, IAA	Sheeja and Mandal, 2003
Manihot esculenta Crantz.	Apical meristem	BAP, Kn	Tang *et al*., 1983
Melia azedarach L.	Hypocotyl segments	BAP, PBA, 2,4-D	Sato and Esquibel, 1995
Momordica charantia L.	Shoot tips	BAP, Kn	Wang *et al*., 2000
Murraya paniculata (L.) Jack.	Shoots	BAP	Jumin and Ahmad, 1999
Narcissus bulbcodium L.	Twin scales	BAP, NAA, IBA	Santos *et al*., 1998
Narcissus triandrus L.	Twin scales of bulbs	BAP, NAA, IBA	Santos *et al*., 2007
Ocimum basilicum L.	Nodes	Kn,BAP, IAA, GA_3, NAA	Sudhakaran and Sivasankari, 2002
Panax ginseng C.A.Meyer	Zygotic embryos, apical and axillary buds	BAP, GA_3, ABA	Lee *et al.*, 1991
Papaver somniferum L.	Callus, green buds	BAP, Kn High illumination and low temp.	Yoshikawa and Furuya, 1983
Pennisetum glaucum L.	Shoot apical meristem	2,4-D, BAP	Devi *et al*., 2000
Pentanema indicum Ling	Shoots	BAP, IBA, IAA	Sivanesan and Jeong, 2007
Perilla frutescens (L.) Britton	Cotyledon explants hypocotyl explants	BAP, IAA, ammonium nitrate,	Zhang, 2007
Petunia hybrida Vilm.	Leaf explants, pedicel	IAA, Zeatin, Kn	Mulin and Thanh, 1989
Phalaenopsis spp.	Nodes	BAP	Duan and Yazawa, 1995
Phalaenopsis hybrida	Nodes	ABA	Wang *et al*., 2002
Phoenix dactylifera var. *deglet* Nour	Juvenile plant	BAP, IAA	Saida *et al*., 1987
Phyllanthus niruri L.	Nodes	BAP, Kn, GA_3	Liang and Keng, 2006
Physalis angulata L.	Apices, nodes	No PGRs	Vasconcellos *et al.*, 2003
Pimpinella tirupatiensis Balakr. et Subram.	Hypocotyl	TDZ, NAA, BAP, GA_3	Prakash *et al.*, 2001
Pisum sativum L.	Shoots	2,4-D, NAA, BAP	Sharma and Kaushal, 2004

Pisum sativum L.	Cotyledonary node and shoot tips	BAP, IBA, NAA, GA_3	Franklin *et al.*, 2000
Polypleurum stylosum (Wight) Hall.	Plant portion	Kn, ABA and water	Sehgal *et al.*, 1993
*Psygmorchis pusilla (*L.)Dodson & Dressler	Plant lets	K, N, Ca, BAP, Glucose and Fructose	Vaz and Ketbauy, 2008
Ptilotus nobili	Nodes	Ethephon, ethylene gas	Parameswara *et al.*, 2009
Ptilotus spicatus Benth.	Nodes	Ethephon, ethylene gas	Parameswara *et al.*, 2009
Pyrus pyrifolia (Burm.) Nak.	Shoot apex and axillary buds	GA_3, GA_4, B-9, CCC S-3307	Higashiuchi *et al.*, 1990
Rauvolfia tetraphylla L.	Nodes, shoot tip	BAP, GA_3	Anitha and Kumari, 2006
Rhododendron spp.	Floral buds/ stamens	TDZ, 2iP	Shevade and Preece, 1993
Ribes nigrum L.	Nodes	GA_3, IBA, Cytokinins,AA, CCC	Schwabe and Al-Doori, 1973
Saccharum officinarum L. var. coc 671	Young leaf rolls	2,4-D, PVP, CM	Virupakshi *et al*., 2002
Salvia africana-lutea L.	Hypocotyl	BAP, NAA, IAA, Kn	Makunga and van Staden, 2008
Saposhnikoviadivaricata (Turez.)Schishk	Root, inter node leaf explants	2,4-D, NAA, BAP, ABA, ETH	Qiao *et al*., 2009
Scoparia dulcis L.	Leaf callus	IAA, BAP, NAA, IBA, Kn, 2,4-D	Annie and Jayachandran, 2008.
Solanum nigrum L.	Node, callus	BAP, IAA, NAA, IBA, 2,4-D	Kolar *et al.*, 2008
Spathoglottis plicata Bl.	Seeds	Kn, NAA	Murthy *et al.*, 2006
Spilanthes acmella Murr.	Shoot tips, node, leaf explants	BAP, NAA, IBA, Kn, 2i P, IAA, GA_3	Saritha and Naidu, 2007b
Streptocarpus nobilis C. B. Clarke	Leaf	BAP, IAA, KNO_3, Sucrose	Simmonds,1982
Streptocarpus nobilis C. B. Clarke	Leaf discs	BAP, Kn, IAA, NAA, 2,4-D	Handro, 1983
Talinum paniculatum (Jaeq) Gaertn.	Protoplasts	2,4-D, NAA, BAP, ZT,	Zhang et al., 1995
Withania somnifera Dunal	Axillary bud	BAP, NAA, Kn, IAA	Saritha and Naidu, 2007a

Ornamental plants

Mulin and van (1989) worked to obtain *in vitro* flowers from thin epidermal cell layers of *Petunia hybrida*. The effect of temperature, day length, photosynthetic photon flux density, ventilation of vessel, cultivars, ambient environments and photosynthesis in the photoperiodic induction of growth and flowering of *Kalanchoe blossfeldianain vitro* were examined (Amaki and Higuchi, 1999; Nell *et al.*, 1982; Yang *et al.*, 1999) respectively. *In vitro* flowering and propagation of *Wahlenbergia stricta* was reported by Carson and Leung (1994b)

There are many workers who worked on the orchids belonging to the genus *Dendrobium*, the *in vitro* flowering was induced in *Dendrobium madame, D. chao- praya-smile* and *D. candidum* by Sim *et al.* (2008), Hee et al. (2007), Wang *et al*. (1990) respectively. Whereas, Banko and Stefani (1991) induced *in vitro* flowering in *Oxydendrum arboretum*. Kerbauy (1984) and Livingston (1962) observed *in vitro* flowering of *Oncidium varicosum* mericlones and *Oncidium pusillum* in flask. In other orchids like *Cymbidium goeringii* and *C. hybridium* the *in vitro* flowering was noticed in the investigations of Li-Ming and Ji-Liang (2006), whereas in *C. ensifolium* var. *misericors* the promotional activity of the cytokinins was tested (Chang and Chang, 2003; Wang *et al*. (1992). So *et al*. (2003) induced *in vitro* flowering in *C.goeringii* by using putative flower induction treatment. Chia *et al.* (1999) reviewed the *in vitro* flowering of orchids. Induction of *in vitro* flowering by the stimulation of cytokinins and gibberellins was studied in *Pharbitis nil* (Galoch *et al.,* 2002), *Begonia* (Ringe and Nitsch, 1968), *Passiflora suberosa* (Scorza and Janick, 1980), *Cymbidum* spp. (Wang, 1990; Wang *et al*., 1992), *Lilium longiflorum* (Wang, 1996) and *Browallia demissa* (Ganapathy, 1969). Several successful attempts to induce *in vitro* flowering of roses have been reported (Vu *et al*., 2006; Wang *et al*., 2002). In *Rosa hybrida*, the flowering was induced by growing nodes on the media fortified with MS + 3 mg/l BAP and 1 mg/l Kn, later it was transferred to 2 mg/l BAP for 9 months (Kachanapoom *et al*., 2009).

Commercial crops

Some recent studies of bamboo species focused on several aspects of *in vitro* flowering, these include *Dendrocalamus latiflorus* (Choun-Sea *et al*., 2007); *D. hookeri* (Ramanayake, 2006), *D. strictus* (Singh *et al*., 2000, Nadgauda *et al.*, (1993) *Bambusa edulis* (Lin *et al*., 2004) and *B. atra* (Ramanayake *et al.,* 2001b).Presence of cytokinins and stress has been attributed for flowering *in vitro* (Ramanayake *et al*., 2001a). John and Nadgauda (1999) induced *in vitro* flowering in Bamboos and reviewed on the same. *In vitro* flowering of albino bamboo (*Bambusa oldhamnii*) regenerants derived from a nine-year old embryogenic cell line was reported by Ho and Chang, (1998). Micropropagation of *B. edulis* through nodal explants of field grown culms and flowering of regenerated plantlets was reported by Lin and Chang (1998) and Lin *et al*. (2003). Joshi and Nadgauda (1997) induced *in vitro* flowers in *B. arundinacea*, Nadgauda *et al*. (1997) compared the *in vitro* bamboo flowering with *in vivo* in *B. arundinacea*. John and Nadgauda (1997) induced *in vitro* flowering in *Bambusa vulgaris* var. *vittata*. *B.arundinacea*. Somatic embryos have also given rise to *in vitro* flowering in *B. vulgaris, Dendrocalamus giganteus* and *D. strictus* (Rout and Das, 1995). Peeters *et al.* (1991) and Hilson and La Motte (1977) induced flower buds in tobacco. Smolders *et al.* (1990) reveals that the dose of NAA determines flower bud regeneration in tobacco explant at a large range of concentration. Naik and Latha (1997) induced *in vitro* flowering in *Morus alba*.

Medicinal Plants

Successful attempts were made on few of the medicinal plants to induce flowering. Vadawale *et al*. (2006), Thiruvengadam and Jayabalan (2001) were able to induce *in vitro* flowering in *Vitex negundo.* The changes of endogenous hormone content during floral bud and vegetative bud differentiation in thin cell layer culture of *Cichorium intybus* explant was studied by Ying-zhang and Bi-wen (1996). In *Phyllanthus caroliniensisin vitro* flowering had been reported by Catapan *et al.* (2000). The maternal and plant growth regulators effect on *in vitro* flowering in *Chenopodium rubrum* and *Chenopodium murale* was studied by Mitrovic *et al.* (2000, 2002). The condition of the apical meristem of seedlings responsive to a promotive effect of abscisic acid on the flowering in the short day plant *Chenopodium rubrum* was studied by Krekule and Kobli (1981) and Seidlova *et al.* (1981). Nitsch and Nisch (1967) induced *in vitro* flowering from the stem segments of *Plumbagoindica.* In *Panax ginseng*, the root callus was used as an explant for the induction of the *in vitro* flowering from the embryoids by Chang and Hsing, (1980) and Tang, (2000). *In vitro* flowering of plantlets regenerated from zygotic embryo derived somatic embryos of Ginseng was reported by Lee *et al.* (1990). The *in vitro* flowering was reported in a valuable medicinal plant *Solanum nigrum* (Jabeen *et al*., 2005). Cvetic *et al.* (2004) induced *in vitro* flowering in *Centaurium pulchellum.* Kintzios and Michailakis (1999) induced *in vitro* flowering from the flower heads of *Chamomilla recutita.* Abdullah *et al.* (2008) studied the effect of different hormones on callus formation and *in vitro* flowering of *Oxalis* sp.

Food crops

In vitro flowering in transgenic *Pyrus communis* was induced by Matsuda *et al.* (2006). *In vitro* flowering and abnormal rooting was found in some antisense shoots in *Pyrus communis* in the investigations of Gao *et al.* (2007). The *in vitro* flowering on long term sub cultured pear shoots was achieved by Harada and Murai (1998). Singh *et al*. (2006) induced *in vitro* flowering in embryogenic cultures of Kinnow mandarin (*Citrus nobilis* × *C. deliciosa*). Tisserat *et al*. (1990) induced *in vitro* flowering from *C. lemon* lateral buds. Influence of temperature and photoperiod of flower induction and inflorescence development in sweet orange (*C. sinensis*) was observed by Moss (1969). Mandal *et al*. (2000) induced *in vitro* flowering in maize (*Zea mays*). *In vitro* pollination fertilization of maize was reported by Higgins and Petolino (1988). Hisajima *et al.* (1987) induced ears from maize seeds *in vitro* and plant regenerated from ovaries of unfertilized ears. Franklin *et al*. (2000) studied the factors effecting *in vitro* flowering and fruiting of green pea (*Pisum sativum*). *In vitro* flowering and pod setting of non-symbiotically germinated pea was reported by Tadashi *et al*. (1999). Pierik *et al.* (1994) tried to find out the relationship between the flowering and position of the axillary buds on the main axis of tomato. Al-Khayri *et al.* (1991, 1992) induced shoots from the callus, which induced flowers on hormone free medium and *in vitro* seed production from sex modified male spinach plants regenerated from callus cultures. *In vitro* flowering of *Murraya paniculata* was induced by Taha (1997). Jumin and Nito (1995, 1996a) induced *in vitro* flowering in orange jessemine (*M. paniculata*), they succeeded in induction of *in vitro* flowers from the protoplasts.Koh and Loh (2000) induced *in vitro* flowering in *Brassica napus*. Vandana *et al*. (1995) induced *in vitro* flowering and pod formation in cauliflower (*Brassica oleracia* var. *botrytis*). Rajasekaran *et al.* (1983) obtained flower formation *in vitro* by hypocotyl explants of cucumber (*Cucumis sativus*).

In some studies on various species aiming at *in vitro* flowering were also studied on a good number of plants, like *Pennisetum glaucum* (Devi *et al*., 2000), *Coriandrum sativum* (Stephen and Jayabalan, 1998), *Vigna mungo* (Ignacimuthu *et al*., 1997), *Vigna radiata* (Avenido and Haulea, 1990), *Solanum tuberosum* (Al-wareh *et al*., 1989), *Amaranthus* (Tisserat and Galletta, 1988), *Glycine max* (Dickens and van Staden, 1985) and in *Helianthus annuus* (Narasimhulu and Reddy, 1984). Ammar *et al.* (1987) induced *in vitro* flowering in *deglet* Nour, a variety in the *Phoenix dactylifera.* A tiny flower containing angiosprmic and high protein processing plant *Wolffia microscopica* was cultured to induce *in vitro* flowering (Maheshwari and Chauhan, 1963).

Rare / threatened plants

Due to over exploitation and lack of organized cultivation of the *Rauvolfia tetraphylla*, the wild population has declined fast and the species is listed as endangered, Sarma *et al.*(1999) studied *in vitro* flowering in this species. The *in vitro* flowering in *Stawellia dimorphantha*, avulnerable plant was studied by Hodgkiss (2004).

Factors effecting in vitro flowering

Flowering is one of the processes that may result in senescence. Hence how ethylene inhibitors can induce flowering is still a mystery. The results of such study will be useful in micropropagation and developmental studies of floral differentiation. The malformation and poor flower quality occasionally observed in the flowers produced *in vitro* may have been at least partially due to competition and or nutritional deficiencies in *Pentanema indicum* (Sivanesan and Jeong, 2007). Factors influencing the growth of micropropagated shoots and *in vitro* flowering of gentian and compared with that of *in vivo* grown plant flowers were studied by Zhang and Leung (2002) respectively.

There are many physico-chemical factors which affected the *in vitro* flowering mechanism. Kolar and Senkova (2008) have reduced the mineral nutrient availability, which accelerated *in vitro* flowering in *Arabidopsis thaliana.* The effect of Paclobutrazole, Light Emitting Diodes (LEDs) and sucrose on flowering of *Euphorbia milli* plantlets *in vitro* was studied by Dewir *et al.* (2007). Lokhande *et al.* (2003) studied the effect of temperature on ascorbate peroxidase activity and flowering of *Arabidopsis thaliana* ecotypes under different light conditions whereas, peroxidase activity in relation to *in vitro* rhizogenesis and precocious flowering in Bamboos was studied by Ansari *et al.* (1996). Important factors for *in vitro* flowering are carbohydrates, growth regulators, light and $_{P}$H of the culture medium (Heylen and Vendrig, 1988). Wada and Totsuka (1982) observed long day flowering of *Perilla* plants cultured in nitrogen poor media. Effects of IAA, zeatin, ammonium nitrate and sucrose on the initiation and development of floral buds in *Torenia* stem segments cultured *in vitro,* was studied by Tanimoto and Harada (1981).Many workers concentrated on the physical factors which played major role in the *in vitro* flowering mechanism. Bernier (1981) reviewed on physiological overview of the genetics of flowering time control. The effects photoperiod and temperature, light intensity and GA_3 on *in vitro* growth and flowering were best observed by Geekiyanage*et al*. (2006), Many factors affected the *in vitro* flowering of *Dendrobium* species, in *Dendrobium moniliforme,* pyroligneous liquor and coconut water influence plantlet multiplication and *in vitro* flowering (Sun-Ok and Dong-Hoon, 2005).

Conclusion

The efforts of many workers, the known information about the applications of *in vitro* flowering and various parameters that effect the growth and flowering in different species has been summarized in this review. So many workers are facing challenges in large scale production of flowers *in vitro,* due to difference in seasonality, lack of knowledge on commercial values, if we overcome these problems in standardizing industrious protocol development for the scarce ornamental and medicinal plants, we can add a new flowers in the bouquet.

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