INTERNATIONAL JOURNAL OF SCIENTIFIC INFORMATION

www.jsiinternational.com

Review Article

In Vitro Flowering in Orchid Species

Sanyogita*
Institute of Biotechnology, Division of Biosciences
University- Mohali, Punjab, India
*Corresponding Author Email: bhartisanyogita9@gmail.com

Article Received on: 27/03/23 Revised on: 03/04/23 Approved for publication: 12/04/23

ABSTRACT

Flowering is an evasive and interesting of all plant's developmental processes. Orchids' relatively lengthy juvenile phase would be cut short, and a deeper understanding of the physiological, genetic, and molecular aspects of flowering would be gained thanks to plants' ability to initiate flowering in vitro. All studies that have been done on orchid in vitro flowering that are currently accessible are included in this review.

Keywords: Dendrobium, orchids, In vitro flowering, Plant growth regulators, Plant tissue culture

INTRODUCTION

When the genetic conditions, such as photoperiod and environmental reactions, are favorable, the plant blooms. (Tissarat and Galletta, 1995). These circumstances can be altered, which will cause the plant to enter the early stages of reproduction. A study following Ribes nigrum reveals that the juvenile-like condition harms the in vitro flowering. (Schwabe and Al-Doori, 1973). The reported attempt to induce flowering in vitro from juvenile explants of some plants also helps to understand the physiology of flowering, which heavily relies on the concentration and interactions of exogenous and endogenous phytohormones, sugars, minerals, and phenolics, among other things. The three main stages are the beginning of floral stimuli, moving them, and floral morphogenesis. There is significant inconsistency in the requirements for plant

growth regulators, according to several studies. plant growth regulators temperature, light regime, and nutritional aspects for *in vitro* flower development in explants from different species (Bernier *et al*., 1981).

In vitro, flowering is crucial for selective hybridization, particularly when using pollen from rare stocks, and it may be the first step towards the possibility of recombining genetic material via in vitro fertilization in lines that aren't otherwise capable of hybridization. Following the first review of in vitro flowering by Scorza (1982), the researchers concentrated on the in vitro flowering of different species. Ramanayake (2006) investigated bamboo species' in vitro blooming. Due to the lengthy adolescent stage of orchids, conventional orchid breeding is a laborious process. Depending on the genotypes, the entire breeding cycle may last 3-5 years. (Hee et al., 2009). When young Dendrobium hybrids were forced to flower in vitro, many employees thrived.

Many workers flourished in the induction of *in vitro* flowering in juvenile *Dendrobium* hybrids (Sim *et al*., 2007; Tee *et al*., 2008). The role of growth regulators in flowering as demonstrated by *in vitro* techniques as outlined by Nitsch (1972).

Factors inducing *in vitro* flowering of orchids

The nature of the signal that induces flowering remains uncertain although physiological studies of the floral transition led to the identification of several floral signals including sucrose, gibberellin, and reduced N-compounds (Corbusier and Coupland 2006). Many factors such as photoperiod, irradiance, temperature, and hormonal regulator affect the *in vitro* flowering in orchids (Chia *et al*. 1999).

PHOTOPERIOD

Photoperiod has a very important effect on *in vitro* flowering of orchids. Long-day

orchids need the minimum light period for flowering and on the other hand, short-day orchids cannot surpass the longest light critical point. Zhu (2006) also reported that flower induction *in vitro* of *Cymbidium* kanran was significantly affected by photoperiod. The percentage of bud initiation progressively condensed, when the light period was continued from 8 h to 14 h while the highest flower bud induction percentage was achieved in an 8-h photoperiod. However, the percentage of bud induction was 28.26% in a 16-h photoperiod, which was higher than that observed in a 12- or 14-h photoperiod, but the induced flower buds did not develop into fully-opened blooms. Jia *et al*. (2000) reported that flower induction *in vitro* of *Cymbidium ensiform* could be attained with continuous light only and never in darkness. Vaz *et al*. (2004) also reported a positive relationship between long days and floral spike formation of

TEMPERATURE

"*Psygmorchis pusilla*." While anthesis was inhibited and flower longevity was decreased, floral bud development was diminished in plant cultivation when the photoperiod was 20 hours or greater. A floral spike was induced, but the flower buds that had been held in darkness did not open, in contrast to the 12- and 16-h photoperiods, which produced perfectly opened flowers. When the photoperiod was changed from 6 to 8 hours and from 22 hours to continuous light, the two main flowering reactions were confirmed. A rise in the number of floral spikes under 8 hours of continuous light may be linked to the carbohydrate build-up seen just before the 6 and 22-hour photoperiods. Pre-existing floral buds that were dormant under short-term conditions may have grown due to the rise in carbohydrate content. In some orchids However, the temperature is used to control flowering time in commercial

cultures of some orchids such as *Cymbidium, Dendrobium, and Phalaenopsis* (Chen *et al.,* 1994; Goh and Arditti, 1985; Hew and Yong, 1997). Nevertheless, the similarities between temperature effects on plants cultivated *in vitro* and *ex*-vitro are not a rule for all species. (Wang *et al*., 2005). *Phalaenopsis amabilis* was grown-up at high temperatures (30/25℃, day/night), flowering was blocked, and this could be reversed by gibberellin A3 or gibberellic acid (GA3) treatment (Wang *et al*., 2005). Likewise, GA3 and temperature influenced carbohydrate content and flowering in *Phalaenopsis,* though no specific details were provided by the authors (Chen *et al*., 1994). Duan & Yazawa (1995a) informed that low-temperature treatments did not promote the formation of floral buds *in vitro* and suggested that the conditions for the induction of floral buds in *Phalaenopsis in vitro* might be different from those *in vivo*. Vaz *et al.* (2004) reported excessive sensitivity to temperature variations by *Psygmorchis pasilla,* 27℃ being the most appropriate for development, leaf and floral spike development. Temperatures of 22℃ and 32℃ were not appropriate for *in vitro* development of *P. pusilla*. High temperatures might have increased respiration rates and lowered CO2 absorption, resulting in carbohydrate depletion, thereby inhibiting growth and delaying flowering.

NUTRITION

Minerals and carbohydrates are the two major factors that affect in vitro flowering. (reviewed by Scorza, 1982; Tee et al., 2008; Ziv and Naor, 2006). In culture media, sugar is regarded as a crucial carbon source for the growth and induction of flowers that will survive. According to Vu et al. (2006), the most suitable sucrose concentration is 30 mg/l, and the presence of NH_4 in the medium was crucial for plant growth and

development. Sucrose was suggested to be the only factor necessary in floral bud induction or initial development while other factors are essential to help them develop completely in the later stages of in vitro floral morphogenesis. Flower development was also enhanced by half the ionic concentration of phosphorus and potassium although the highest Calcium concentration shows some floral stimulation. Plants grown under nutrient-deficient conditions were understandably more yellow than those cultured on full-strength medium.

PLANT GROWTH REGULATORS

Auxins and Cytokinins

In vitro flowering studies of orchids, a single PGR (plant growth regulator) such as BA, 2iP, TDZ, ABA, GA3, TIBA (2,3,5-triiodo benzoic acid) or NAA, and combinations of other PGRs and nutrients were used to induce *in vitro* flowering (Chang and Chang, 2003; Duan and Yazawa, 1994a; Kostenyuk *et al*., 1999; Sim *et al*., 2007; Te-Chato *et al*., 2009; Wang *et al*., 2009). BA has been used for the maximum *in vitro* flowering studies of several orchids, together with *Dendrobium primulinum Lindl*. (Deb and Sungkumlong, 2009), Doriella Tiny (Duan and Yazawa, 1994a), *Dendrobium Chao* Praya Smile (Hee *et al*., 2007), *Cymbidium nive*omarginatum Mak (Kostenyuk et al., 1999. *Bulbophyllum auricomum* seed, with the highest percentage (50-15.8%) of flowering observed in MS medium. The role of CKs in floral evocation might be in governing early mitotic activity, precocious initiation of axillary meristems, and increased rate of appendage production by the meristems (Scorza and Janick, 1980). Similarly, CK was cited as a probable component of a multi-factored flowering stimulus (Bernier, 1988). BA was essential for the normal *in vitro* development of rose floral buds (Vu *et al*., 2006). which

probably measured floral development through genes controlling shoot apical meristem movement (Lindsay *et al*., 2006).

Gibberellin

An appropriate amount of GA is essential for flowering to occur, and the deficiency in the GA biosynthetic pathway increases the photoperiodic sensitivity. And a high concentration of GA3 suppressed flower bud induction and flourishing.

Abscisic acid and Ethylene

Many explanations suggest that the 8 of ABA is inhibitory to flowering *in vitro* (Vaz and Kerbauy, 2008b). Exposure to ethylene is capable of inducing flowering in the species. Flowering frequency was amplified by pre-treatment of proto corms in the ABA-containing medium followed by transfer into the medium (Wang *et al*., 1997). Anthesis was favored by the removal of ethylene using a $KMnO_4$-trap (Vaz and Kerbauy, 2000).

CULTURE METHODS

Sim *et al.* (2007) described the use of a two-layered medium (liquid over Gelrite-solidified) to promote the initiation of inflorescence stalks, flower buds, and flower growth from protocorms of the *Dendrobium Madame Thong-In*. and volume of the liquid medium also affected flower bud formation and its development. This method was successfully applied to induce *in vitro* flowering of another *Dendrobium* hybrid, Chao Praya Smile (Hee *et al*., 2007). But, in D. Second Love, shoots could be induced to produce flowers *in vitro* after culturing in a Phytagel-solidified medium with TDZ (Ferreira et al., 2006). Hence, the physical state of the medium i.e. fluid or Gelrite-solidified medium, and probably air exchanges played an important role in *vitro* flowering.

CONCLUSION

The efforts of many scientists and the identified evidence about applications of *in vitro* induction or flowering and several

restrictions that affect the growth and flowering of different species of orchids have been summarized in this review paper. Many workers are facing problems in the large-scale production of flowers *in vitro*, due to differences in seasonality, and a lack of information on commercial values, if we overcome these problems we can add new flowers to the garland.

References

Bernier, G., Kinet, J.M. and Saches, R.M. The physiology of flowering, Boca Raton, CRC Press, v.1: 1-19. (1981). Chang C, Chang WC. Cytokinins promotion of flowering in Cymbidium ensiform var. misericords in vitro. Plant Growth Regul, 39, 217–21(2003).

Chen WS, Liu HY, Liu ZH, et al.. Gibberellin and temperature influence carbohydrate content and flowering in

Phalaenopsis. Physiol Plant, 90, 391–5. (1994) Chen WS, Liu HY, Liu ZH, et al.. Gibberellin and temperature influence carbohydrate content and flowering in

Phalaenopsis. Physiol Plant, 90, 391–5(1994) Chia, T. F., Arditti, J., Segeren, M. and Hew, C.S.. Review: in vitro flowering of orchids. Lindleyana 14:60-76. (1999) Demeulemeester, M.A.C. and De Proft, M.P.. In vivo and in vitro flowering response of chicory. Influence of plant age and vernalization. Plant Cell Rep. 18: 781-785(1999)

Ferreira WM, Kerbauy GB, Kraus JE, et al.. Thidiazuron influences the endogenous levels of cytokinins and IAA during flowering of isolated shoots of Dendrobium. J Plant Physiol, 163, 1126–34(2006)

Hee KH, Loh CS, Yeoh HH. Early in vitro flowering and seed production in culture in Dendrobium Chao Praya Smile (Orchidaceae). Plant Cell Rep, 26, 2055–62.

Hee KH, Loh CS, Yeoh HH. (2007). Early in vitro flowering and seed production in culture in Dendrobium Chao Praya Smile (Orchidaceae). Plant Cell Rep, 26, 2055–62. (2007).

Hee, K.H., Hock Hin Yeoh. and Chiang Shiong, L.O.H. In vitro flowering and in vitro pollination: methods that will benefit the orchid industry, In: Proceedings of NIOC 2009, Nagoya, Japan(2009).

Jia YJ, Cao YL, Wang, B, et al.. Studies of the culture of internode of flower branch of China orchid. J Sichuan Uni (Nat Sci Edition), 37, 94–7(2000)

Knudson L.. A new nutrient solution for the germination of orchid seeds. Am Orchid Soc Bull, 15, 214–7(1946) Kostenyuk I, Oh BJ, So IS. Induction of early flowering in Cymbidium niveo-marginatum Mak in vitro. Plant Cell Rep, 19, 1–5(1999)..

Kostenyuk I, Oh BJ, So IS. Induction of early flowering in Cymbidium niveo-marginatum Mak in vitro. Plant Cell Rep, 19, 1–5(1999).

Lindsay DL, Sawhney VK, Bonham-Smith PC. Cytokininduced changes in CLAVATA1 and WUSCHEL expression temporally coincide with altered floral development in Arabidopsis. Plant Sci, 170, 1111– 7(2006)..

Nitsch, C.. The role of growth regulators in flowering as demonstrated by in vitro techniques. Proceedings Advdvanced Stududies, lzmir Institute: 413-421. (1972) Ramanayake, S.M.S.D.. Flowering in Bamboo: An enigma!Ceyl. J. Sci (Bio Sci.) 35:95- 105(2006)

Schwabe, W. W. and Al-Doori, A.H. Analysis of a juvenile like condition affecting flowering in the black currant (Ribes nigrum). J. Exp. Bot. 24: 969-981. (1973). Scorza R.. In vitro flowering: a review. HortScience, 4, 106–27. (1982)

Scorza, R. In Vitro flowering, In: Janick, J (Ed). Horticultural Reviews, 4: 106-127(1982)

Sim GE, Loh CS, Goh CJ.High frequency early in vitro flowering of Dendrobium Madame Thong-In (Orchidaceae). Plant Cell Rep, 26, 383–93. . (2007). Tisserat, B. and Galletta, P.D. In vitro flowering and fruiting of Capsicum frutescens L. Hort. Sci. 30:130132(1995).

Vaz APA, Figueiredo-Ribeiro RCL, Kerbauy GB.. Photoperiod and temperature effects on in vitro growth and flowering of P. pusilla an epiphytic orchid. Plant Physiol. Biochem, 42, 411–5. (2004)

Vaz APA, Figueiredo-Ribeiro RCL, Kerbauy GBPhotoperiod and temperature effects on in vitro growth and flowering of P. pusilla an epiphytic orchid. Plant Physiol. Biochem, 42, 411–5. (2004)

Vaz APA, Kerbauy GB. Effects of mineral nutrients on in vitro growth and flower formation of Psygmorchis pusilla (Orchidaceae). Acta Hortic, 520, 149–56(2000).

Vaz APA, Kerbauy GB. In vitro flowering studies in Psygmorchis pusilla. In: Teixeira da Silva JA, ed. Floriculture, Ornamental and Plant Biotechnology: advances and Topical Issues (1st Edn, Vol. V). Isleworth, UK: Global Science Books, Ltd., Chapter 42, 421–6. . (2008b)

Vu NH, Anh PH, Nhut DT. The role of sucrose and different cytokinins in the in vitro floral morphogenesis of Rose (hybrid tea) cv. ‘‘First Prize’’. Plant Cell Tiss Organ Cult, 87, 315–20. (2006).

Vu NH, Anh PH, Nhut DT. The role of sucrose and different cytokinins in the in vitro floral morphogenesis of Rose (hybrid tea) cv. ‘‘First Prize’’. Plant Cell Tiss Organ Cult, 87, 315–20. (2006).

Wang GY, Xu ZH, Chia TF, Chua NH. In vitro flowering of Dendrobium candidum Sci China (Sr. C),40,35-42 (1997).

Wang YQ, Du L, Wang SQ. Research development of flowering control of Phalaenopsis. Northern Hortic, 3, 34-6 (2005).

Cite this Article as: *Sanyogita. In vitro Flowering in Orchid Species. Int. J. Sci. Info. 2023; 1 (1): 24-32.*

Source of support: Nil, Conflict of interest: None Declared