A review on *in vitro* haploid production in orchids

Abstract

The aim of writing this paper is to review "*in vitro* haploid production in orchids."Haploids possess half number of chromosomes and do not undergo fertilization. Haploid plants are produced from haploid culture. *In vitro* conditions provide the necessary nutrients and conditions required for the growth of a haploid plant. Orchids are the most favourite as well as the most expensive cut-flowers. They are commercially very valuable. Due to the high market value of Orchids, there would be pressure on the cultivators to produce a large number of Orchids. The only hurdle in this process is the natural breeding program of Orchids. The natural breeding cycle of Orchids is very slow as well as unpredictable. To reduce this time, the technique of *in vitro* haploid production is used. *In vitro* conditions can decrease or shorten the time required for juvenile period in Orchids. This technique is useful to produce homozygous pure lines and to increase the yield of a particular plant.

Key-words: *In vitro*, haploid production, orchidaceae, orchid seeds, angiosperms, monocots.

Introduction

Family Orchidaceae contains very beautiful and vibrant flowers. Orchids are found almost all over the world, except in the freezing Polar Regions and extremely hot deserts (Oo *et. al.*, 2021).Orchids are mostly found in tropical forests (Oo *et. al.*, 2021). The flowers of the orchidaceae family are of different shapes and radiant colours (Sarmah *et. al*., 2017). Orchidaceae is a very interesting family because of the colours of its flowers and the exceptional structures of the flowers (Oo*et. al*., 2021). These flowers are commercially very useful. Orchids are flowers that have high artistic value. Orchids are endowed with economic worth at par with other ornamentals (Sipayung *et. al.*, 2018).

Orchidaceae contains roughly 35,000 species, in 1,000 genera and 1,00,000 hybrids(Vendrame*et. al.*, 2014). Orchids are around 8% to 10% of all flowering plants (Vendrame *et. al*., 2014).The annual orchid flowers production in India is approximately 1000 tonnes makinga small share of 0.01% in international market (Sarmah *et. al.*, 2017; Chugh *et al.*, 2009). Orchids are the most expensive cut- flowers. Besides their ornamental value, some genera also provide food products and medicines (Vendrame *et. al.*, 2014). The

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commercial value for Orchids is very high, due to which they are in high demand all over the world. The commercial demand for Orchid plants is increasing day by day, So the improvements in Orchid cultivation and their conservation can effectively develop the economy of our country.

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***In vitro* anther, microspore ovary and ovule culture**

Growing Orchids naturally is a time-consuming and slow process (Mishra and Goswami, 2014).Orchids has a long vegetative phase before they flower (Kaur and Bhutani, 2013). The breeding cycle of Orchidaceae is also very slow and unpredictable (Teixeira da Silva *et al.*, 2013).To reduce or minimize this time, the technique of *in vitro* haploid production is used. Haploids are useful in the shortening of the breeding cycle in Orchids (Hu, 1997). This technique is really helpful for the development of various species of Orchids. Haploids are widely used in many agricultural and horticultural fields (Pickering and Devaux, 2016). By using *in vitro* technique, we can develop haploid as well as diploid plants. In this review paper, the production of haploid plants is being discussed.

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Haploid plants are those plants that arise from gametes or gamete-like cells (Britt and Kappu, 2016). They do not undergo fertilization and can still generate a viable individual (Britt and Kappu, 2016). Haploid plants contain cells that have half the number of chromosomes. Haploid plants are sterile as they contain only one set of chromosomes number (Basu *et al.*, 2011).Haploids have only one chromosome set, that is formed after the meiosis in male or female gametes (Britt and Kappu, 2016). The plant is referred to as the maternal or paternal, based upon the set of chromosomes contributed bypaternal or maternal side (Britt andKappu, 2016). The haploid plant is also known as monoploid because it contains only one set of chromosomes (Basu *et al.*, 2011).There are many advantages of haploid plants such as fixation of homozygosity, shorten breeding cycle, and high selection efficiency (Mishra and Rao, 2016).Haploid plants are used in the basic plant research fields such as cytogenetics, molecular genetics, crop evolution, plant biotechnology, and traditional plant breeding (Toureav *et al.*, 2009).Haploid plants are an effective tool for induced mutagenic and genetic transformation studies (Folling and Olesen, 2002).*In vitro* techniques are an efficient way to produce haploids than other methods such as interspecific hybridization or treatment with plant growth regulators, temperature, or irradiation for haploid production (Atanassov *et al.*, 1995).*In vitro* methods of haploid production include-

Androgenesis which is the production of haploids by the anther or pollen culture. In androgenesis, the haploid is generated from the male gamete (Mishra and Goswami, 2014). Androgenesis is of two types-Direct androgenesis and indirect androgenesis including Parent plant selection, Selection of flower bud, microspore development stage determination, surface sterilization of young flower buds, Isolation of anther/microspore from buds, inoculation of anther on an appropriate medium and regeneration and recovery of the plant (Mishra and Goswami, 2014).Gynogenesisis the production of haploids by the ovary or ovule culture (Mishra andGoswami, 2014). In gynogenesis, the female gametes are used for the production of the haploid plant. The common steps for gynogenesis include selection of the healthy parent plant, checking pf ovary/ovule developmental stage by histology, selection of appropriate flower bud and surface sterilized, inoculation of ovary/ovule in a suitable medium, incubation of ovary/ovule culture, regeneration of callus/embryo and haploid plant recovery (Mishra andGoswami, 2014).

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The *in vitro* culture technique was first given by Haberlandt in 1902. *In vitro* conditions in haploid production effectivelyreduce the juvenile period (Teixeira da Silva *et. al*., 2013; Blanchard and Runkle, 2008; Taylor and Staden, 2006). This technique is also called tissue culture, organ culture, and cell culture (Samrah *et. al.*, 2017). Good quality and disease-free Orchid plants can be obtained by using this technique (Sarmah *et. al.*, 2017).

The desired plant can be selected from any forest or garden. The collected plant is then replanted in a pot filled with soil (Kaur and Bhutani, 2013). This plant is kept under *in vitro* conditions. The *in vitro* conditions provide the environment that promotes the growth of the plant (Kaur and Bhutani, 2013).*In vitro* conditions comprise a proper physical and chemical environment (Atanassov *et. al.*, 1995).

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The developmental stage of microspores is crucial. Androgenesis is the production of haploid microspore and their *in vitro* culture. This is only possible with the immature anther containing immature pollen (Atanassov *et. al.*, 1995). Juvenility has important role to play in regeneration during androgenesis (Datta and Wenzel, 1998).Pre-treatment of anthers are the most efficient approach to the anther culture involves a change in temperature (Atanassov*et. al.*, 1995).Cold pre-treatmentskillsnon-viable microspores and anthers (Atanassov *et. al.*, 1995). It blocks the microspores in the first mitosis. Thus, change the development from gametophytic phase to sporophytic phase and these pre-treatments maintain the cellular environment which are conducive for embryogenic development (Atanassov *et. al.*, 1995).

Culture Media is a complex solution that provides suitable conditions for cell growth. Several culture mediums are present. Different media are suitable for different plant species such as Murashige and Skoog (MS) medium (Atanassov *et. al.,* 1995), Knudson (KC) medium (Kaur and Bhutani, 2013), Vacin and Went medium (Kaur andBhutani, 2013), Linsmaier and Skoog (LS) medium (Saad and Elshahed, 2012) and Nitsch and Nitsch (NN) medium (Atanassov *et. al*., 1995).Among all of these media, MS medium is the most commonly used medium. The salt composition of any medium does not affect the frequency of initial division, it affects the planting efficiency a little, but it enhances embryogenesis and regeneration process (Atanassov *et. al.,* 1995). The sugar content in media also shows several effects. A high concentration of sugar is crucial for inducing androgenesis (Atanassov *et. al*., 1995).The proper growth of a plant is also affected by the growth regulators, carbon source, and growth additives. The growth regulators play an essential role in deciding the regeneration pathway to be followed by explant in nutrient regime (Hussain *et. al.,* 2012).

The success of *in vitro* production depends upon the right culture conditions such as temperature and light. The ideal conditions for *in vitro* haploid production are different for different species (Atanassov *et. al.,* 1995). Anthers are kept under dark conditions with a temperature of 20- 30°C (Atanassov *et. al.,* 1995). The optimal temperature for the field grown material is usually 2°C which is higher than the greenhouse-grown material (Atanassov *et. al*., 1995). *In vitro* Microspore culture is all about culturing immature pollen grains that are obtained by the donor plant (Rajcan*et. al.,* 2011).The methodology of microspore culture is similar to that of the anther culture (Rajcan*et. al*., 2011). This method is slower than the anther culture method for development, but the production of the embryo is higher in this method (Atanassov *et. al.,* 1995).

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The method of *in vitro* haploid production has now been adopted in various plant reproducing programs world over. The methodology has emerged as an efficient technique for prompt harvest improvement for gene transfer, part of chromosome,and whole chromosomes (Baenziger and DePauw, 2009; Basu*et. al.,* 2011; Ceoloni and Jauhar, 2006;Touraev*et. al.,* 2009).Being economically stable technique, it is effectively utilised in biodiversity conservation (Sarmah *et. al.,* 2017). Thus, *In vitro* haploid is avery productive and advisable way for haploid production (Mishra and Goswami, 2014). Haploid plants have several applications in the fields of basic research, classical plant genetics, cytogenetics, genetic

transformation research, conventional plant breeding studies, genome mapping, and many more (Basu*et. al.*, 2011).

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CONCLUSION

Orchids are the most beautiful and commercially very valuable flowers. They are very expensive. The improvement in orchid cultivation caneffectively develops the economy of our country. The natural breeding cycle of Orchids is very long and time-consuming. It takes several years for orchids to blossom. *In vitro* conditions can effectively shorten the time required for the breeding cycle. *In vitro* haploid production can also be used to develop viable seedlings ofrare, endangered, and commercially valuable Orchids. *In vitro* haploid production raises good-quality, orchid plant free of disease. Success of this technique is based upon identifying the right stage of the capsule at harvesting time, the growth adjuvants, and suitable nutrient regime. *In vitro* haploid production is very useful in developing homozygous pure lines, such pure lines are utilised in various plant breeding programs, and in the research activities pertaining to plant genetics and breeding.

Comment [DGAS13]: plants

References

1. Atanassov, A.,Zagorska, N., Boyadjiev, P., andDjilianov, D. (1995). *In vitro* production of haploid plants. *World Journal of Microbiology & Biotechnology*, 11(4), 400-408.
2. Baenziger, P.S., DePauw, R.M. (2009). Wheat breeding: Procedures and strategies. In: B.F. Carver (ed.) Wheat: Science and Trade. *Wiley-Blackwell Publishing*, Ames, IA.
3. Basu, S. K., Datta, M., Sharma, M., Kumar, A. (2011). Invited Review Article Haploid production technology in Wheat, and some selected higher plants. *Australian Journal of Crop Science*, AJCS 5(9), 1087-1093, ISSN: 1835-2707.
4. Blanchard, M.M., Runkle, E.S. (2008). Benzylamide promotes flowering in *Doritaenopsis and Phalaenopsis*Orchids. J Plant Growth Regul, 27, 141-150.
5. Britt, A. B., Kappu, S. (2016). Cenh3: An Emerging Player in Haploid Induction Technology. Front. Plant Sci. 7: 357. Doi: 10.3389/fpls.2016.00357.
6. Ceoloni, C., Jauhar, P.P. (2006). Chromosome engineering of the Durum Wheat genome: Strategies and applications of potential breeding value. In: Singh, R.J. and Jauhar, P.P(eds.) Genetic resources, chromosome engineering, and crop improvement. CRC Press/Taylor and Francis, Boca Raton, FL. Vol 2: Cereals. P. 27-59.

7. Chugh S., Guha S and Rao (2009) Micropropagation of Orchids: A review on the potential of different explants. Sci Hortic 507-520.
8. Datta, S.K., Wenzel, G. (1998). Single microspore derived embryogenesis and plant formation in Barley (*Hordeum vulgare*), Arch Zeucht, pp. 125-131.
9. Folling. L., Olesen. A. (2002). Transformation of Wheat (*Triticum aestivum* L.), microspore-derived callus and microspores by particle bombardment. Plant Cell Rep. 20: pp. 629-663.
10. Hu. H. (1997). *In vitro* induced haploids in wheat. *In vitro* haploid production in Higher Plants, 73-97. Doi: 10.1007/978-94-017-1862-2_3.
11. Hussain. A., Qarshi. I. A., Nazir, H., Ullah. I. (2012). Plant tissue culture: Current status and opportunities, Recent advances in plant *in vitro*culture. Leva, A.,Rinaldi, M. R., L. IntechOpen, DOI: 10.5772/50568.
12. Kaur. S., Bhutani K.K. (2013). *In vitro* propagation of *paphiopedilum spicerianum*(*Reichb. F.*)*Pfitz.* Floriculture and Ornamental Biotechnology7 (1), 65-70 © Global Science Books.
13. Mishra. V. K.,Goswami. R. (2014). Haploid Production in Higher Plant.IJCBS REVIEW PAPER VOL_1 (ISSUE 1), ISSN- 2349-2724.
14. Mishra. R.,Rao. G. (2016). *Invitro* Androgenesis in Rice: Advantages, Constraints, and Future prospects. Rice Science, 23(2), 57-68.
15. Oo. T. Z., Oo. K.T., Hlaing. N.Z. (2021). *In vitro* Regeneration of White Dancing Lady Orchid (*Oncidium alliance*).
16. Pickering. R.A., Devaux. P. (2016). Haploid production: approaches and uses in plant breeding, 519-547.
17. Rajcan, Boersma. J. G., Shaw. E. J. (2011). Plant Genetics Techniques: Plant Breeder`s Toolbox. Comprehensive Biotechnology (Second Edition). Volume 4, Pages 133-147.https://doi.org/10.1016/B978-008-088504-9.00252-X.
18. Saad. A. I.M., Elshahed. A. M. (2012). Plant Tissue Culture Media. Recent Advances in Plant *in vitro* Culture. Leva, A., M. R. Rinaldi, L. IntechOpen. DOI: 10.5772/50569.
19. Sarmah. D., Kolukunde. S., Sutradhar. M., Singh. B., Mandal. T., and Mandal. N. (2017). A Review on: *In vitro* Cloning of Orchids. *International journal of current microbiology and applied sciences*, 6(8), 1909-1927.
20. Sipayung. P., Matanari. J., Lafau. M., Sulastri. Y., Ginting. B., andSihombing. D. (2018). The effect of activated charcoal dose and benzyl amino purine concentration on the

growth of Orchid plantletsin Murashige and Skoog media *in vitro*. IOP ConferenceSeries: Earth and Environmental Science, 205, 012025.

21. Taylor.N.J.,Staden. J. Van (2006). Towards an understanding of the manipulation of *in vitro* flowering. In: Teixeira da Silva, J.A., ed. Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues (1st ed., Vol. IV). Isleworth, UK: Global Science Books, 1-22.
22. Teixeira.Da. Silva. J.A., Kerbauy. G. B., Zeng. S.,Chen. Z., and Duan. J. (2013). *In vitro* flowering ofOrchids.*Critical reviews in Biotechnology*, 34(1), 56-76. https://doi.org/10.3109/07388551.2013.807219.
23. Touraev. A., Forster. B.P., Jain. S.M. (2009). Advances in haploid production in higher plants. *Springer Science and Business Media B.V*., The Netherlands. P. 1-208.
24. Vendrame. W.,Faria. R., Sorace. M., and Sahyun. S. (2014). Orchid cryopreservation. *Ciencia E Agrotecnologia*, 38(3), 213-229.