International Journal of Innovative Research and Review ISSN: 2347 – 4424
Online, International Journal, Available at http://www.cibtech.org/jirr.htm
2022 Vol.10, pp.8-13/Prem and Amala
***Review Article** (Open Access)*

A REVIEW ON RAPID MULTIPLICATION OF MEDICINALLY IMPORTANT *BOERHAAVIA DIFFUSA*

***Prem Kumar. A and Amala Elizabeth Joseph**
Department of Biotechnology, Loyola College, Chennai, India
**Author for Correspondence*

ABSTRACT
Boerhaavia diffusa L is a medicinal herb with immense pharmaceutical significance. The plant is used by many herbalist, Ayurvedic and pharmaceutical industries for production of biopharmaceuticals. It is among the 46 medicinal plant species in high trade sourced mainly from wastelands and generally found in temperate regions of the world. In this review article, we highlight the importance of multiple shooting and callus induction through several explants using Murashige and Skoog (MS) medium supplemented with hormones like BAP, 2, 4 D under *in vitro* condition in *Boerhaavia diffusa.*

***Key Words**: Boerhaavia diffusa, Axillary Buds, Explants, Regeneration, Hardening*

INTRODUCTION

Plants have been used for medicinal purposes long before prehistoric period. Ancient. Evidence exist thatUnani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products.

Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs (Zahid, 2016). These herbs are easy to grow, look good, taste and smell amazing and many of them are magnets for bees and butterflies. Many herbs are used as blood purifiers to alter or change a long-standing condition by eliminating the metabolictoxins. These are also known as 'blood cleansers'. Certain herbs improve the immunity of the person, thereby reducing conditions such as fever.

Boerhaavia diffusa is found as a weed during rainy season in India, Northern and South American continents and South Eastern Africa. *Boerhaavia diffusa* L commonly called *rakt punarnava* is herbaceous member of the Nyctaginaceae family. There are two varieties of these plants, one with white flower known as *shweta punarnava* (*Boerhaavia erecta*) while other with red flower called as *rakta punarnava* (*Boerhaavia diffusa*) out of which *rakt punarnava* (*B. diffusa*) is mainly used for medicinal purposes. Out of the 40 species of *Boerhaavia* genus, five are found in India – *B. diffusa, B. chinensis, B. erecta, B.rependa,* and *B. rubicund* (Patil *et al.*, 2015). Seeds of Boerhaavia diffusa show a very low germination rate and vegetative propagation is limited. The plant possesses pharmaceutically important chemical constituents such as punarnavine, eupalitin, boeravinones A-H, β-sitosterol and β-sitosterol-β-D-glucoside. It is among the 46 medicinal plant species in high trade sourced mainly from wastelands.

Boerhaavia diffusa is a medicinal plant of Nyctaginaceae family. It is commonly called Punarnava because of its regeneration property in rainy season. It is creeping perennial weed, prostrate herb and can grow upto 1m long with spreading branches. The flowers are small in size and pink in colour in small umbels and arranged in slender, axillary and terminal panicules. The stem is prostrate, woody or succulent, cylindrical and often purplish, hairy and thickened at its nodes. Mass propagation of plant through *in vitro* culture is one of the best method of commercial application of plant tissue culture technology. Increasing demand for medicinal plant is one of the major cause for their depletion from

natural habitat. Micro propagation offers a great potential for conservation and large scale multiplication of such useful species. A large number of publication have been made on its chemistry, pharmacological effect etc. but a little attempt has been made on its *in vitro* regeneration. Mass collection of this plant fromits habitat lead to depletion of this species (Mishra, 2015.).

Medicinal Importance of Boerhaavia diffusa

Boerhaavia diffusa contains many bioactive compounds like tannins, flavonoids, alkaloids, terpenoids, phenolic compounds, glycosides, rotenoids, steroids etc. It has a wide range of properties like anti- inflammatory, diuretic, anticonvulsant, antistress activity, antioxidant activity, antiantherosclerotic activity and hyper tension reducing activity, antibacterial, antidiabetic, antiasthmatic. It is used to treat diseases like jaundice, skin troubles, kidney problem, inflammation, eye diseases, wounds, reproductive system (Mishra *et al.*, 2014). The work is focused on an efficient regeneration with lesser duration and lesser requirement of plant growth regulators for achieving higher frequency of shoot multiplication of *Boerhaavia diffusa* with potential active ingredients.

Root extracts were used as an adjuvant in treatment of pulmonary tuberculosis, chemotherapy and very potent source of alkaloid drug punarnavine (Pandey *et al.*, 2019). Two rotenoids isolated from roots reported to contain a ribosome-inactivating protein BDP-30 (Srivastava *et al.*, 2015).These bioactive compounds are responsible for treating number of ailments like cancer, diabetes, inflammation etc.

Boerhaavia diffusa is used in traditional medicines, now its pharmacological effect is validated by number of scientific experiments.

B. diffusa is an exclusive or important constituent of several ayurvedic preparations. BD is a good source of nutritional supplements as reported 15 amino acids (6 essential) in the whole plant and 14 amino acids (7 essential) in the roots along with isopalmitate acetate, behenic acid, arachidic acid, and saturated fatty acids. The presence of vitamins C, B3, and B2 along with calcium in roots. In various tribal areas, its roots as well as whole plant have been reported to be used as culinary ingredient (Mishra *et al.*, 2014).

Boerhaavia diffusa is an important herbal constituent of many ayurvedic formulations. It is used in the treatment of inflammation, jaundice, asthma, rheumatism, nephrological diseases, asthma and gynecological disorders.*Boerhaavia diffusa* has a long history of indigenous uses by tribal people and in ayurvedic or natural herbal medicines. The whole plant is a very useful source of drug punarnava. It contain several secondary metabolites like flavonoid glycosides, rotenoids, steroids, alkaloids, phenolic and lignan glycosides. The studies have showed that it has several pharmacological properties and used in formulations meant for inflammation, rheumatism, neurological disorders, jaundice, asthma, anaemia and gynaecological disorders (Mishra *et al.*, 2014). *B. diffusa* has a great demand by the local herbalist and ayurvedic companies. The roots and leaves with flowers have been found to be highly potent. It is very effective to treat seminal weakness and blood pressure. In ayurvedic medicine, root and aerial parts of thisplant were reported for the treatment of diabetes. It is also used in the treatment of stomachache, anemia etc. (Kaur, 2019). It has been used in several parts of India as it can cure several diseases like asthma, urinary disorders, jaundice etc. It has several other pharmaceutical properties like anti-cancerous, anti- HIV, anti-hepatitis.

In Vitro Regeneration of Boerhaavia diffusa

Tissue culture-based technology is widely accepted for clonal propagation and ensures consistent regeneration of this species. This technology provide option to study germination, storage and viability of artificially developed germplasm, particularly for those species. For industrial uses, several tons of *Boerhaavia diffusa* has been collected from different parts of India, as a consequence of unsustainable collection it is feared that this plant species become severely depleted or become extinct. *Ex situ* conservation and development of suitable agro-technology cultivation are the two

means by which species can be made available in the future. *In vitro* culture protocol is not only used to propagate endangered species but also for accelerating selection process but also enhance the efficiency of desirable traits at the cellular level in crops. *Boerhaavia diffusa* is propagated by seeds but the seed viability is very low and germination rate is low. Under such condition it is very important to develop techniques for rapid and mass propagation of these species to meet up the commercial needs and to protect them depletion from nature. Mass scale collection of this plant by local herbalists and ayurvedic companies from natural habitat lead to depletion of this species. Under such conditions, it is important to develop techniques for rapid and mass propagation of this species to meet up the commercial needs and also for protecting the species from depletion from nature. *In vitro* micro propagation is proven to be reliable and efficient method for mass propagation of rare and endangered species. Few attempts have been made for *in vitro* regeneration of *Boerhaavia diffusa* using leaves and stem as explants.

Callus Culture of Boerhaavia Diffusa

Plants develop unorganized mass of cell called callus. Exogenous application of auxin and cytokinin induce callus in various plants. The induction of callus involves many factors like adjustment of culture medium, type of explant and condition of maintenance of *in vitro* culture.

With 2, 4 D as plant growth regulator, leaf explant showed better callus formation than stem explants. The best result in terms of percent response and dry weight basis were obtained at 2mg and induction period was 8 days and the obtained callus was friable and opaque in nature (Jain, 2003). MS basal medium supplemented with BAP+2, 4 D induced callus indifferent cultures after 3-4 weeks of inoculation (Joshi, 2015).

MS medium supplemented with 2, 4 D showed best callus proliferation of 78%. MS medium supplemented with kinetin showed 45.2% callus induction (Mishra, 2015.). For callus induction leaf explants were cultured on MS medium supplemented with different concentrations of 2, 4 D.

Shoot Induction in Boerhaavia diffusa

Maximum shoot proliferation occur on a medium containing BAP (Benzyl amino purine) and NAA (Naphthalene acetic acid). BA exhibited better response than kinetin in shoot bud proliferation.

Proliferation of multiple shoots were obtained was obtained with high frequency from nodal segments and shoot tips within three days of inoculation. These explants were capable of directly developing multiple shoots on MS medium containing different concentration and combinations of Cytokinin, BAP and Kinetin. Highest percentage of shootlets formation and maximum number of shootlets per explant were obtained in MS medium supplemented with 1.5mg/l of BAP (Wesley, 2010).

Flower development from *in vitro* regenerated shoot on MS medium supplemented with NAA and BA. *In vitro* flowers are similar to flowers in *in vivo* plants. BA supplemented with 5% coconut milk is effective for flowering and multiple shoot formation. Age of explants affect flowering. Mature explants high probability of flowering compared to juvenile explants (Chervathur *et al*., 2015).Root formation is found on explants transferred to MS medium supplemented with IAA. Micro propagation facilitates rapid multiplication of this important and valuable medicinal plant. It reduce intensive labour and shorten time period for development of plantlets.

Table 1: Different explants and hormones used for callus culture of *B. diffusa*

S. No.	Explant	Hormones	Reference
1.	Leaf	BAP, Kinetin, NAA,IAA	Mishra, 2015
2.	Leaf	2,4 D, BAP	Pandey *et al*., 2019
3.	Leaf	2,4 D	Wesley *et al*., 2010

Root Formation From in Vitro Grown Plants

Rooting of *in vitro* regenerated shoots were achieved in MS medium enriched with IAA. The root initiation time, number of roots and the length of roots were recorded. The rooted plantlets obtained through micropropagation is transferred from culture medium to sterile soil. The percentage survival is also recorded.

Table 2: Different explants and hormones used for shoot induction of *B. diffusa*

SI NO	Explants	Hormones	Reference
1.	Shoot apex, Nodal explants	IAA,NAA,BAP, Kinetin	Majumder *et al*., 2021
2.	Leaf	BAP,NAA	Mishra, 2015
3.	Nodal explants	2,4 D, NAA	Pandey *et al*., 2019
4.	Nodal explants	BAP	Patil *et al*., 2015
5.	Nodal explants	BAP,NAA	Dasgupta *et al*., 2013
6.	Leaf, shoot-tip, nodal intermodal explants	BAP, Kinetin, NAA	Wesley *et al*., 2010
7.	Nodal explants	Kinetin , IAA	Ray *et al*., 2008

CONCLUSION

Large scale multiplication of elite clone of *Boerhaavia diffusa* can be achieved from axillary buds. Rapid shoot induction can be induced on media supplemented with BAP. Callus induction can be achieved using 2, 4 D hormone. This review is helpful in rapid regeneration of healthy clone of *Boerhaavia diffusa*for commercial production and *ex situ* conservation.

ACKNOWLEDGEMENT

I thank Department of Biotechnology and Loyola College Management for providing the facilities for thestudy.

REFERENCES

Anita J (2015). Effect of Hormone on Callus Induction in *Boerhaavia diffusa* L. *International Journal ofAllied Practice, Research and Review* **2**(3*)* 57-61.

Ashu P, Oshin V and Suresh C (2019). *In vitro* propagation of *Boerhaavia diffusa* L.: An important medicinal plant of family Nyctaginaceae. *Indian Journal of Genetics and Plant Breeding* **79**(1) 89-95.

Avik R and Sabitha B (2008). *In vitro* propagation of *Boerhaavia diffusa* L.: An important medicinalplant of family Nyctaginaceae. *International Journal of Plant Developmental Biology* **2**(2) 123-127.

Babita A, Sunanda D and Archana P (2011). *Boerhaavia diffusa* Linn: A Review on its Phytochemicaland Pharmacological Profile. *Asian Journal of Applied Sciences,* **4** *663-684.*

Battu GR, Dakoju RS and Devarakonda R (2018). Qualitative Physicochemical and Phytochemical Analysis and Quantitative Estimation of Total Phenols, Flavonoids and Alkaloids of *Boerhaavia diffusa*.*Journal of Global Trends in Pharmaceutical Sciences.* **9**(1) 4823-4832.

Biswas A, Bari MA, Roy M and Bhadra SK (2009). Clonal Propagation through Nodal Explant Culture of *Boerhaavia diffusa* L. - A Rare Medicinal Plant. *Plant Tissue Culture and Biotechnology* **19**(1) 53-59.

Goyal BM, Bansal P, Gupta V, Kumar S, Singh R and Maithani M (2010). Pharmacological Potentialof Boerhaavia diffusa: An Overview. *International Journal of Pharmaceutical Sciences and Drug Research* **2**(1) 17-22.

Harpeet K (2019) *Boerhaavia diffusa*: Bioactive Compounds and Pharmacological Activities. *Biomedical Pharmacology Journal* **12**(4) 1675-1682.

Juna BGR, Sugunan VS, Suhara BS (2017). Neutraceutical Evaluation of *Boerhavia diffusa* L. *International Journal of Current Pharmaceutical Research* **9**(1) 101-104.

Juna BGR, Suhara BS, Sugunan VS (2014). Nutritive and anti-nutritive properties of *Boerhavia diffusa* L. *Journal of Pharmacognosy and Phytochemistry***. 2**(6) 146-151.

Kapil SP and Sanjivani RB (2015). Efficient micropropagation and assessment of genetic fidelity of *Boerhaavia diffusa* L- High trade medicinal plant. *Physiology and Molecular Biology of Plants* **21** 425-432.

Manal AS (2014). Highly Efficient *In Vitro* Regeneration Method of Endangered Medicinal Plant *Heliotropium kotschyi* (Ramram) in the Kingdom of Bahrain. *American Journal of Plant Sciences* **5**(5) 736-747.

Mahesh AR, Harish K, Ranganath MK and Raviraj AD (2012) Detail Study on *Boerhaavia diffusa*
Plant for its Medicinal Importance-A Review. *Research Journal of Pharmaceutical Sciences* **1**(1) 28-36.

Rajendra S, Anita S, Madan MS, Amla B (2011). Impact of Phytohormones in Micropropagation ofMedicinally Potent Plant: *Boerhaavia diffusa* L. *International Journal of Pharmaceutical Sciences Review and Research* **8**(1) 85-89.

Rakhi S, Daman S, Bilikere SD, Madhu C (2011). Inhibition of Human Cervical Cancer Cell Growth by Ethanolic Extract of *Boerhaavia diffusa* Linn. (Punarnava) Root. *Evidence-Based Complementary andAlternative Medicine.*

Richa B, Ankita Y and Ra S (2014). Phytochemicals and Antioxidant Activity in *Boerhavia diffusa*. *International Journal of Pharmacy and Pharmaceutical Sciences*. **6**(1) 344-348.

Roy PK (2008). Rapid Multiplication of *Boerhaavia diffusa* L. Through *In vitro* Culture of Shoot tip andNodal Explants. *Plant Tissue Culture and Biotechnology* **18**(1) 49-56.

Shikha M, Vidhu A, Praveen KG and Sanjay MJ (2014). Phytochemical, Therapeutic, and Ethnopharmacological Overview for a Traditionally Important Herb: *Boerhavia diffusa* Linn. *Biomed Research International*.

Sumita D and Reddy MN (2003).Clonal propagation of *Boerhaavia diffusa* Linn. – A Valuable Medicinal Plant. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **4**(3).

Sweety M, Animesh B, Mohammad MR (2021). *In vitro* propagation and Cytological Analysis of *Boerhaavia diffusa* L-An Important Medicinal Plant Species of Bangladesh. *American Journal of PlantSciences* **12** 1347-1360.

Thakur M K and Sulekha P (2015). Phytochemical, Nutritional and Antimicrobial Properties of *Boerhaavia Diffusa. International Journal of Science and Research* **5** 400-404.

Tulika M (2015).*In Vitro* Studies of *Boerhaavia diffusa* Linn. *International Journal of Current Research*
7(5) 15563-15566.

Vandana J, Singh D, Swarnalata S and Saraf S (2003). *In Vitro* Culture Studies for Callus and RootGeneration of *Boerhaavia diffusa* Linn. *Ancient Science of Life*. **22**(4) 134-139.

Venkatajyothi R (2017). *In-vitro* Anticancer activity of *Boerhaavia diffusa* Linn. *International Journalof Current Research in Biology and Medicine.* **2**(3) 20-24.

Wesley EG, Johnson M, Regha P and Kavitha S (2010). *In vitro* propagation of *Boerhaavia diffusa* L.through direct and indirect organogenesis. *Journal of Chemical and Pharmaceutical Research* **2**(5) 339-347.