



A Mini Review On Plant Tissue Culture Of Amla (*Phyllanthus Emblica*)

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Abstract:

Amla, also known as Indian gooseberry or Usiri in Telugu, is a tree native to India and surrounding countries. It's highly valued in traditional Indian medicine (Ayurveda) and is also popular in other traditional medicine systems such as Siddha and Unani. Amla is scientifically known as *Phyllanthus emblica*. Amla is exceptionally rich in vitamin C, containing significantly more vitamin C than oranges. It also contains various other nutrients, including vitamin A, calcium, potassium, and antioxidants. Amla is widely used in traditional medicine for its various health benefits. It is believed to improve digestion, boost the immune system, promote hair health, and enhance skin health. It is also used in the treatment of various ailments such as diabetes, respiratory disorders, and liver problems. Despite its sour taste, Amla is used in various culinary preparations in India. It is commonly used to make pickles, preserves, chutneys, and candies. It is also used to make Amla juice, which is consumed for its health benefits. Amla is a common ingredient in hair care products due to its purported benefits for hair health. It is used in hair oils, shampoos, and conditioners to promote hair growth, prevent premature graying, and improve hair texture. Amla plant propagation presents challenges due to its slow growth, low seed germination rates, and vulnerability to pests and diseases, necessitating meticulous care and attention during cultivation. Amla plant tissue culture holds significance in agriculture as it offers a controlled environment for mass production of disease-free and genetically identical plants, ensuring higher yield and quality. Additionally, it facilitates the preservation of rare or endangered amla varieties and enables the rapid propagation of elite cultivars with desirable traits, contributing to sustainable agriculture and conservation efforts. Thus, this concise review provides insights into the tissue culture of the Amla plant. It begins by elucidating the morphological characteristics, chemical compositions, and diverse applications of *Phyllanthus emblica*. Subsequently, the chapter delves into the protocols and methodologies for culturing Amla in vitro, highlighting the intricacies of tissue culture conditions. Finally, the review underscores the significance of tissue culture in Amla plant propagation, emphasizing its pivotal role in overcoming traditional propagation challenges and ensuring the sustainable cultivation of this valuable botanical resource.

Key words: Amla, *Phyllanthus emblica*, *Emblica officinalis*, Tissue culture, Explant.

Introduction:

Phyllanthus emblica (L.) (synonym *Emblica officinalis* Gaertn.), also known as 'Aonla,' 'Amla,' or Indian Gooseberry, is a member of the Euphorbiaceae and Geraniales order. The plant could be referred to as the "Mother of herbs" because of its effective therapeutic properties and significant medicinal and therapeutic demand (Goyal and Bhadauria. 2008) As per ancient Indian mythology, the Amla tree was the very first tree to emerge on Earth. (Pria et al. 2019). It is a deciduous tree that is indigenous to India,

Indonesia, and the Malay Peninsula, as well as China, Myanmar, Sri Lanka, and Pakistan (Thilaga et al. 2013; Walia et al. 2015). The trees are found in tropical, subtropical, and coastal areas of India, along with hill cliffs and mountains (up to 200m and 4500ft., respectively), and are farmed in plain and hilly areas of Kashmir valley. (Rai et al. 2012)

Amla is a subtropical woody fruit-bearing tree that can be propagated in a variety of climatic conditions. The tree is grown in tropical and dry subtropical climates for effective large-scale cultivation. The tree requires 630-800mm of annual rainfall and can withstand temperatures as high as 46°C (warm temperature suitable for fruit growth). Because the tree is deep-rooted and deciduous, any type of soil is suitable for its growth. The plant can also survive over arid or semi-arid climatic conditions. *E. officinalis* has a huge potential for growth in high saline areas and, to a lesser extent, ravine land. According to Ayurvedic literature, the fruit has the following properties of rasa (flavor), veery (cooling), and vipaka (digestive). It is widely used in treatment of inflammation, fever and general burning sensations in the body (Williamson, 2002). Amla has proven efficacy as anti-diuretic, febrifuge, and in hairloss control in the Tibetan system of treatment (Tsarong and Tsewang, 1994). In India, Amla fruits are largely used in anti-pyretic and anti-inflammatory medications (Ihantola-Vormisto et al. 1998; Srivasuki, 2012). The fruit is known to be rich in a wide array of active ingredients like alkaloids, tannins, amino acids, glycosides, phenolic acids and terpenoids (Zhang et al. 2000a, b, 2001a,b,c, 2002, 2003; Subramanian et al. 1971; Krishnaiah et al. 2009). The fruits juice of Amla is reported to have maximum content of vitamin C as compared to other fruits like lime, apple, grapes and pomegranate (Jain et al. 2004; Tarwadi and Agte, 2007). Different plant parts are rich in various phytochemically active ingredients (Zhang et al. 2001a,b,c).

Propagation of Amla (*Embllica officinalis*) poses challenges due to its hard seed coat hindering germination, low success rates in cutting propagation due to lignified stems, and the requirement of patience and skill in air layering for root formation despite its reliability (Jalal et al. 2019). Tissue culture is best choice for over come the difficulties in natural propagation method. Tissue culture offers a viable alternative to overcome the challenges encountered in natural propagation methods for Amla, providing a reliable means to efficiently propagate the plant while bypassing issues such as low germination rates and difficulty in rooting cuttings.

Tissue culture is vital for Amla plant propagation due to its ability to rapidly multiply disease-free plants, facilitate genetic improvement, ensure year-round production, and conserve genetic resources in a controlled environment, thus overcoming the challenges of traditional propagation methods. Therefore, this review aims to provide insights into the application of tissue culture techniques for Amla plant propagation and cultivation.

***Phyllanthus emblica*:**

Phyllanthus emblica Linn, a member of the euphorbiaceae family, is extensively distributed throughout the majority of tropical and subtropical nations. Phyllanthus is a very large genus containing approximately 550–750 species and 10 to 11 subgenera. It is endemic to equatorial southeast Asia and is found in the mixed forest of tropical and subtropical regions at elevations between 150 and 1,400 m. In

Indonesia *P. emblica* is called balakka, kimalaka, kemlaka, kemloko, or malaka (Summanen, 1999; Mal and Meena, 2022). Natural products have existed since the dawn of humanity, the significance of traditional systems of medicine and particular traditional medical practices is now acknowledged worldwide. To evaluate selective pharmaceuticals of herbal origin, it is now necessary to adopt an intelligent and pragmatic approach.

Application of *Phyllanthus emblica*

All parts of *Phyllanthus Emblica*, including its fruits, flowers, seeds, leaves, and bark, have been extensively utilized in numerous traditional remedies. Pharmacological studies reveals that *P. emblica* have antioxidant (Chaphalkar et al., 2017; Sheoran et al., 2019), anticancer (Ngamkitidechakul et al., 2010; Chekdaengphanao et al., 2022; Naik and David, 2023), Immunomodulatory (Jantan et al., 2019), cytoprotective (Zhang et al., 2016), anti-viral (Lv et al., 2015), anti-jaundice, anti-dyslipidemic (Quranayati et al., 2023), anti-aging (Wu et al., 2022), anti-apoptotic (Chekdaengphanao et al., 2022), anti-inflammatory (Wang et al., 2019), hepatoprotective (Pramyothin et al., 2006), nephroprotective (Huang et al., 2023), and anti-diabetic (Naik and David, 2023).

Chemical constituents in *Phyllanthus emblica*

Phyllanthus emblica has various constituents have been used in the formulation of numerous herbal and patent medicines (Dinesh et al., 2017). The majority of fixed oils, essential oils, and phosphatides are found in fruit seeds. Fruit, leaves, and bark are rich in tannins; bark also contains leucodelphinidin, while roots are abundant in lupeol and ellagic acid (Hussain et al., 2021). The yellowish-brown seeds of *P. emblica* contain linoleic acid, stearic acid, palmitic acid, linolenic acid, myristic acid, and oleic acid while D-myoinositol, D-fructose, and D-glucose are predominantly found in the ethanol fractions of *P. emblica* (Saini et al., 2022). *Phyllanthus emblica* contains chemopreventive compounds like lupol and glochidone, which belong to the lupine-type triterpenoids (Ramasamy et al., 2012). Additionally, it harbors antioxidant properties from compounds like mallotusin, isomallotusin, isostrictinin, and mallonin, along with phyllembilin, cinnamic acid, and chebulagic acid (Ahmad et al., 2021). The vitamin C content in *P. emblica* far surpasses that of common citrus fruits like lemons, oranges, and tangerines (Bajgai et al., 2006). Besides vitamin C, this fruit also contains other essential vitamins including carotene, niacin, riboflavin, and thiamine (Ghosal, 1996). For every 100 g of *P. emblica* fruit, there is an impressive vitamin C quantity ranging from 600 to 1,300 mg. Among the amino acids present, the prominent ones are glutamic acid (29.6%), proline (14.6%), aspartate (8.1%), alanine (5.4%), and lysine (5.3%) (Saini et al., 2022). *Phyllanthus emblica* fruit is rich in vitamin C (70%–72%) and includes a variety of components such as tannins, phembembaic acid (6.3%), gallic acid (5%), lipids (6%), emblicol, flavonoids, and mymic acid. The leaves of *P. emblica* contain gallic acid, chebulic acid, ellagic acid, kaempferol, kaempferol-3-o-glucoside, gallo tannin, and rutin, phosphoric acid, essential oils, linoleic acid, oleic acid, stearic acid,

palmitic acid, and mystic acid. The bark of the plant contains proanthocyanidins, tannins, and leucodelphinidin. Moving on to the roots, they contain ellagic acid and lupeol (Saini et al., 2022). This thorough review focuses on the phytochemical makeup and the effects of *P. emblica*. Through an extensive exploration, this study aims to shed light on the numerous health advantages of *P. emblica*, thereby stimulating more research and progress in utilizing this herbal remedy to enhance human health.

Invitro propagation of *Phyllanthus emblica*:

In vitro propagation ensures the rapid multiplication of plantlets from plant cells and tissues on nutrient media under aseptic conditions (Mukherjee et al. 2019). Conventionally, *E. officinalis* is propagated through seeds and asexually by budding and grafting. Propagation through seeds is not beneficial since seeds possess dormancy and do not produce true-to-type plants owing to cross-pollination and seed-derived plants bear inferior quality fruits (Mishra et al. 2011). To overcome this issue, micropropagation techniques were employed to produce large-scale true-to-type and disease-free plants. Several methods of in vitro propagation have been executed in *E. officinalis* to date. This review compiles different approaches attempted for micropropagation and developmental work done in *E. officinalis*

Explants used for the tissue culture of *Phyllanthus emblica*

A proper selection of explant is important in any micropropagation study. The stage, regenerating ability and preferably disease-free were taken into consideration while selecting explant (Mukherjee et al. 2019). A number of explants such as nodal segments, shoots, hypocotyls, epicotyls, embryo, root–shoot node, and leaf have been used by researchers for both direct and indirect regeneration in *E. officinalis* (Table 1). Nodal segment and shoots are generally used for direct organogenesis (Verma and Kant 1999; Mishra and Pathak 2001; Mishra et al. 2006; Goyal and Bhadauria 2008; Patidar et al. 2010). Preferably, the 10–15th nodal segment portion was taken as they gave better response than the young nodal segment because they cannot withstand the disinfection process and older segment showed lower response because of mature tissues (Mishra and Pathak 2001). For indirect organogenesis via callus formation, cotyledons, hypocotyls, epicotyls, embryo, and leaf are being used (Sehgal and Khurana 1985; Gupta et al. 1994; Verma and Kant 1999; Al-Sabah et al. 2012; Priyanka et al. 2014; Thilaga et al. 2013; Priyanka and Singh 2015). Hypocotyls, epicotyls, and zygotic embryos are found to be more promising than leaf for indirect somatic embryogenesis. Other explants such as root and root shoot nodes were also reported to be used (Gour and Kant 2009).

Table 1: Tissue culture of different explants of Amla

Plant Species	Culture part (Explant)	Reference
<i>Phyllanthus emblica</i>	Nodal segments and leaves	Gautam et al. 2021
<i>P. emblica</i>	Leaves, stems, and roots	Unander,1996
<i>Phyllanthus emblica</i>	Seeds	Singh and Sharma, 2015
<i>Emblica officinalis</i>	Nodal segments	Patidar et al 2010
<i>Emblica officinalis</i>	Nodal segment, cotyledons, cotyledonary nodes, hypocotyls	Verma and Kant, 1999
<i>Emblica officinalis</i>	Axillary/ nodal shoot	Mishra and Pathak (2001)
<i>Emblica officinalis</i>	Root and Root nodes	Gour and Kant (2009)
<i>Emblica officinalis</i>	Zygotic embryo, Leaf	Thilaga et al. (2013)
<i>Emblica officinalis</i>	Shoots	Mishra et al. (2006)

Medium used for tissue culturing of *Phyllanthus emblica*:

The basal medium serves as the source of both macronutrients and micronutrients to the explant under in vitro condition (Gantait and Panigrahi 2018). It also provides vitamins and other organic components required for the growth and development of plants. Almost all researchers have used Murashige and Skoog (MS) (Murashige and Skoog 1962) medium for various experiments in *E. officinalis* (Sehgal and Khurana 1985; Gupta et al. 1994; Verma and Kant 1999; Mishra and Pathak 2001; Mishra et al. 2006; Goyal and Bhadauria 2008; Gour and Kant 2009; Patidar et al. 2010; Nayak et al. 2010; Al-Sabah et al. 2012; Madharia and Dutta 2012; Priyanka et al. 2014; Thilaga et al. 2013; Priyanka and Singh 2015). Many have reported that half-strength MS medium was effective for rooting in *E. officinalis* (Verma and Kant 1999; Mishra et al. 2006; Gour and Kant 2009; Nayak et al. 2010; Al-Sabah et al. 2012). The use of liquid MS medium in full-strength was found to be effective especially during the root induction of in vitro shoots (Sehgal and Khurana 1985). In table 2 has given information about media and plant growth regulator used for tissue culture of *Phyllanthus emblica*

Table 2: Media and plant growth regulators used for tissue culture of Amla.

Plant	Media and PGR	References
<i>Phyllanthus emblica</i>	MS Medium with 2,4-D, BAP (PGR)	Gautam et al. 2021
<i>Phyllanthus emblica</i>	α -naphthaleneacetic acid (NAA) and 3.0% sugar.	Singh and Sharma, 2015
<i>Phyllanthus emblica</i>	MS medium with 5 BAP + 0.5 NAA (PGR)	Verma and Kant (1999)
<i>Emblica officinalis</i>	Modified MS medium with 0.4 Kn + 1 GA3 (PGR)	Mishra and Pathak (2001)
<i>Emblica officinalis</i>	MS medium with 4 BAP + 0.5 NAA	Patidar et al. (2010)
<i>Phyllanthus emblica</i>	BAP+GA3, 2,4-D	Unander,1996

Note: 2,4-d, 2,4 dichlorophenoxyacetic acid; BAP, N6-benzylaminopurine; GA3, gibberellic acid; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; Kn, kinetin; MS, Murashige and Skoog; NAA, α -naphthalene acetic acid; NM, not mentioned; PGR, plant growth regulator

Conclusion:

The Amla plant, scientifically known as *Emblica officinalis*, holds a revered place in various cultures for its wide range of applications. Traditionally, Amla has been used in Ayurvedic medicine for its numerous health benefits, including boosting immunity, improving digestion, and promoting hair and skin health. Its high vitamin C content makes it a potent antioxidant, aiding in the prevention of various diseases and promoting overall well-being. Despite its myriad uses, the traditional propagation of Amla poses several challenges. Amla seeds have a hard seed coat, making germination difficult and often resulting in low success rates. Additionally, propagating Amla through cuttings is challenging due to the lignified nature of its stems, which inhibits root formation and hampers the establishment of new plants.

In addressing these difficulties, tissue culture emerges as a pivotal solution. Tissue culture techniques involve the growth of plant cells or tissues under sterile conditions in a nutrient-rich medium. This method offers several advantages for Amla propagation: **Rapid Multiplication:** Tissue culture enables the rapid multiplication of Amla plants, allowing for the production of a large number of genetically identical clones within a short period. **Disease-Free Plants:** Through tissue culture, Amla plants can be propagated from sterilized plant material, resulting in disease-free stock. This helps in maintaining the health and vigor of the plants and reduces the risk of disease transmission through propagation. **Genetic Improvement:** Tissue culture facilitates genetic improvement of Amla plants by providing a controlled environment where specific traits such as yield, fruit quality, and resistance to diseases can be enhanced through selection and manipulation of plant tissues. **Year-Round Production:** Tissue culture enables year-round production of Amla plants,

irrespective of seasonal limitations. This ensures a continuous supply of planting material for growers, contributing to increased productivity and profitability.

In conclusion, while the Amla plant boasts diverse applications and benefits, its traditional propagation methods are fraught with challenges. Tissue culture emerges as a crucial technique for overcoming these difficulties, offering a sustainable and efficient means of propagating Amla plants while ensuring their health, genetic integrity, and continuous availability for cultivation and utilization.

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