A STUDY ON MICROPROPAGATION OF *Phyllanthus amarus* (Phyllanthaceae): PRESENT AND FUTURE SCOPE

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Abstract: The plant *Phyllanthus amarus* is commonly known as Keezhkainelli and used in various systems of medicines as excellent hepatoprotective agent. This review describes the enhancement of secondary metabolites of *Phyllanthus amarus* through tissue culture or micro propagation. MS media was supplemented with various enhancing materials such as growth promoters, phytohormones, precursors, abiotic elicitors etc., This study aims to review the studies conducted on various methods of micro propagation of *Phyllanthus amarus* and enhancing its secondary metabolite contents.

***IndexTerms - Phyllanthus amarus*, Tissue culture, Secondary metabolites, Abiotic elicitors.**

I. INTRODUCTION:

There is a complex relationship occur between people and plant in our joint evolutionary history. Nowadays plants provide nutrition, fiber, pharmaceuticals and energy for animals and people. Plants are central to our well being, not only as food but also as key components of our cultures, religious and medicines. In olden days, indigenous forest people collect plant materials for medicinal use. Nowadays the whole plant or parts of the plant (stem, leaves, seeds, fruits, bark, root and rhizome) is used as medicines in different formulation in various system of medicine such as Ayurvedha, Siddha, Homeopathy (ASH) system of medicine etc. For example, in Ayurvedha the whole plant of *Catharanthus roseus* used in the treatment of tumors, Senna leaves used as laxative, Amla fruits have good antioxidant properties, rhizomes of turmeric used for benign tumor etc.

Liver is the heaviest gland and second largest organ of human body. The primary function of liver includes detoxification, energy production, energy storage, nutrients conversion, immunity, hormonal balance, digestion, fat regulation etc. During detoxification process, the liver suffers from hepatotoxicity / liver dysfunction. Liver dysfunction is a condition in which liver is not working efficiently and/or is overloaded with toxics or slugs. Most of the herbal drugs are prescribed for this hepotoxicity. Among these drugs, *Phyllanthus amarus* act as excellent hepatoprotective agent and also used in the treatment of jaundice and liver cirrhosis.

Phyllanthus amarus has got hepatoprotective properties, antioxidant & anti inflammatory properties (Kiemer et al. 2003) and also antiviral properties against hepatitis B virus and reverse transcriptase of retroviruses (Shead et al. 1992 and Thyagarajan et al. 1988) and used as remedies for many conditions such as dysentery, influenza, vaginitis, tumours, diabetes, diuretics, malaria, gonorrhea, asthma, diuretics, kidney stones, dyspepsia, ulcer and urinary disorders. The plant is also useful for treating hepatotoxicity, hepatitis B and other hepatic disorders (Rajasri and Sabita Bhattacharyya 2001).

The hepatoprotective activity of *Phyllanthus amarus* has been reported due to the presence phyllanthin and hypophyllanthin. The plant has highly valuable hepatoprotective agent but it suffers from the problem of short lifespan (July to October) due to its low herbage, availability in limited duration, stringent requirement of climatic condition (that is damp weather) for growth and leading to uncertainity in stable supply throughout the year. Due to these problem natural development or conventional propagation of the crop limits the all round availability (Janifer et al. 2012).

In biotechnology, tissue culture techniques are widely used for the invitro synthesis of secondary metabolites and their large scale production. Micro propagation is one of the tissue culture techniques of great importance in mass multiplication of plants invitro. Scarcity in availability of plant material source can easily be overcome by micro propagation or clonal propagation.

This review discusses elicitation as an approach for enhanced production of secondary metabolites of *Phyllanthus amarus*. An 'elicitor' may be defined as a substance which, when introduced in small concentrations to a living cell system, initiates or improves the biosynthesis of specific compounds. Elicitation is the induced or enhanced biosynthesis of metabolites due to addition of trace amount of elicitors.

II. PLANT PROFILE:

Synonyms:

Phyllanthus fraternus, Phyllanthus carolinianus Blanco, Phyllanthus humilis Salisb, Phyllanthus filiformis Pav.ex Baill, Phyllanthus microphyllus Mart.nom.illeg, Phyllanthus purpurascens Kunth, Phyllanthus parvifolius stend.

Vernacular names (Jay Ram Patel et al. 2011**)**:

Tamil	:	Keezha nelli
Malayalam	:	Keezhar nelli
Telugu	:	Neala usirika
Kannada	:	Nela nelli
Sanskrit	:	Bhumyamalaki
Hindi	:	Jangli amla, Bhuyiavla
Other names	:	Gale of the wind, Seed under leaf, Stone breaker, carry me seed

Taxonomical classification:

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Malpighiales
Family	:	Phyllanthaceae
Genus	:	*Phyllanthus*
Species	:	*amarus*

Fig 1: Pattern of growth of *Phyllanthus amarus*

Habit and habitat:

Phyllanthus amarus is a monoecious erect annual herb which grows up to one meter height. Stems are often branching and have 3-10 cm long (can reach up to 15cm) usually with 20-42 leaves. The leaves are alternate, membranous, oblong or elliptic oblong. It has 3-8 mm long; 2-5 mm wide. The petiolate, petioles are very short (0.2 – 0.7mm long). It is membranous or thinly papery; base rounded, apex obtuse or rounded and often apiculate, stipulate, lanceolate, scarious, acute 0.8 – 1 mm long. Leaves are dark green above and paler and grayish beneath. Seeds are light brown or yellowish brown and about 0.9mm long, triangular with 6-7 longitudinal ribs and many transverse striations on the back (Bagchi et al. 1992). The flowering and fruiting season is July to October month.

Chemical constituents:

Securinine, nor-securinine, dihydrosecurinine, geraniin, corilagin, 1,6-digalloylglucopyranoside rutin, quercertin, isoquercertin, lignans (Kassuya et al.2006, Srivastava et al.2008, Leite et al.2006 and Maciel et al. 2007), (niranthin, demethylenedioxy-niranthin, phyllanthin and hypophyllanthin, nirtetralin, isolintetralin) phytol, amariin (Foo 1993), amarulone (Rao and Bramley 1971), amarinic acid, alkaloids like epibubbialine, sobubbialine (Joseph and Raj 2011), fatty acid, saponin, palmitic acid and oleic acid.

Fig 2: Photograph showing the seeds arranged underneath the leaf in *Phyllanthus amarus*

Distribution of *Phyllanthus amarus*:

Phyllanthus amarus is mainly found in Amazon rain forest and other tropical & sub tropical areas such as South East Asia, Southern India, China, Pakistan, Malaysia, west Africa etc.,

III. TISSUE CULTURE ON *PHYLLANTHUS* SPECIES:

Nodal segments are used for micro propagation of *Phyllanthus stipulatus* (Euphorbiaceae). MS media (Murashige and Skoog 1962) supplemented with either 2.5-5.0 mM IBA, maximum multiplication rate (8-9 shoots per explant) was achieved. MS media with 0.62 mM BA was the best basal media for axillary shoot. In the absence of growth regulators, 100% rooting was achieved. Under *ex vitro* conditions, 88% of regenerated plantlets are survived. After 12 weeks of acclimatization, flowering was observed in 81% of the *ex vitro* grown plantlets. Root cultures were successfully established on MS medium containing 1.1 mM NAA (Elizabete catapan et al. 2001).

For axillary shoot proliferation, nodal segments are used for micro propagation of *Phyllanthus urinaria* (Euphorbiaceae). Maximum multiplication rate was achieved when MS media supplemented with 1.0 µM BA. 100% rooting was achieved in MS medium with 2.0 µM IBA. Under ex vitro conditions about 80–90 % of regenerated plantlets survived (Kalidass and Mohan 2009).

IV. PLANT TISSUE CULTURE WORK DONE ON *Phyllanthus amarus*:

For assessing fruit or seed germination in vitro and ex vitro, dried fruits (undehisced seeds) of *Phyllanthus niruri* accessions were collected from 3 different localities such as Greater Accra (Kwabenya), Central (Kasoa) and Eastern (Aburi) regions of Ghana. The seeds from 3, 5 or 7 days dehisced fruits were selected and nursed (ex vitro) in the soil substrate suggesting that there was fruit wall imposed dormancy. Seeds germinated from 7 days dehisced seeds having the highest percentage (68.8%) germination. To improve percentage germination, dehisced seeds were cultured on Murashige and Skoog (1962) medium supplemented with 0-1.2 mg/l BAP or kinetin. At these treatments, MS medium supplemented with 1.2 mg/l BAP had the highest percentage (61.1%) of seed germination when compared to kinetin (Adusei-Fosu et al. 2012).

Leaf bits and internodes are used as explants for regeneration of *Phyllanthus amarus*. Maximum callus induction was obtained on Murashige and Skoog basal medium supplemented with NAA and 2,4-D. The obtained callus was repeatedly sub-cultured for four cycles at 3 weeks intervals. When the callus was sub-cultured on MS medium with BAP (1.0 mg/l) and glycine (50 mg/l), high frequency of callus proliferation was obtained. 87.09% of rooting was achieved on half strength MS medium supplemented with IBA (0.5 mg/l -1) and IAA (0.5 mg/l -1) (Chitra et al. 2009).

For invitro regeneration of *Phyllanthus niruri*, nodal segments were used as explants. MS media supplemented with 1 mg/l Benzyladenine (BA) was chosen as the best medium for induction of multiple shoot. Absence of multiple shoots formation

in MS media with 0.5 or 1.0 mg/l kinetin without BA and highest number of multiple shoots produced in the media supplemented with 1.0 mg/l BA without kinetin. Add kinetin into MS media with presence of BA which induces multiple shoot formation in all (100%) nodal segments. The media supplemented with gibberellic acid (0.5 mg/l) produce in vitro flowering (within 1 week) but invitro fruiting was inhibited. After 4 weeks of culture on the media with 30g/l sucrose, all the invitro plantlets produce flowers and fruits which are similar to that of mother plants (Ong Poh Liang and Chan Lai Keng. 2006).

Shoot tips and single node explants are used for micro propagation of *Phyllanthus amarus* due to its antiviral property. The shoots are elongated in MS medium containing BAP & IAA. The micro propagation study showed that shoot tips are the best source of explants for multiple shoot induction. The elongation of invitro developed shoots was better in a medium supplemented with BAP and IAA and poor response produced in medium with BAP alone [5]. For micro propagation of *Phyllanthus amarus* Schum & Thonn, shoot tips are used as explants and MS medium supplemented with various concentrations of growth hormones. After 2 weeks of sub cultures, media with BAP alone shows poor response and average number of shoots found more in medium with 0.1 mg/L BAP and 0.05 mg/L NAA. MS media with IBA (1.0mg/L) was suitable for rooting (Ravindhran et al. 2006).

For regeneration of *Phyllanthus niruri*, nodal segments are used as explants and MS medium supplemented with different concentrations and combinations of plant growth regulators. The highest frequency (85.5%) of bud formation and shoot induction was observed on medium supplemented with 1.0 mg/l BAP along with 0.5 mg/l IBA. MS medium fortified with 1.0 mg/l BAP and 1.0 mg/l Kinetin in combination with NAA (0.5 mg/L) showed the highest percentage (80.0%) shoot multiplication. Maximum number of roots (85%) was produced in MS medium with 1.0 mg/l IBA. After acclimatization, about 98% of plantlets are survived (Aarti Patel et al. 2018).

V. ENHANCING SECONDARY METABOLITES THROUGH TISSUE CULTURE:

For culturing of *Phyllanthus niruri* MS media, Gamborg B_5 media and White's media supplemented with different concentrations of phytohormones are used. Flowers and young leaves are used as explants. The study was revealed that MS and Gamborg media were found to be suitable for induction of callus culture and MS media supplemented with hormones 2, 4-D (1.0 mg/l) and Kinetin (0.5 mg/l) showed maximum percentage induction of callus and also maximum amount of phyllanthin (0.805%) (Prashanth Kumar et al. 2012).

Suspension culture of *Phyllanthus niruri* yielded 2 newer lignans such as 1.Cubebin dimethyl ether and 2.Urinatetralin but which is reported earlier in *Phyllanthus urinaria*. Media supplemented with precursors such as 0.5 mM ferulic acid or 0.5 mM caffeic acid, resulted in an increasing the lignans 1 and 2 (Elfami et al. 2006).

In immobilized cell cultures of *Phyllanthus amarus*, MS medium supplemented with different phytohormones and precursors such as kinetin, napthalene acetic acid, chitosan and cinnamic acid solution. It was reported that enhancement of phyllanthin and hypophyllanthin was dependent on precursor concentration. Cinnamic acid treatment gave maximum yield of hepatoprotective bioactives as compared to other precursor and phytohormones used (Thakur and Kharya 2011).

Immobilized sodium alginate beads of *Phyllanthus amarus* leaves were used and MS medium supplemented with different abiotic elicitors such as chitosan, copper sulphate, phenylalanine and silver nitrate solution. It was revealed that based on elicitor concentration secondary metabolites of *Phyllanthus amarus* was increased. Silver nitrate (low concentration) treatment gave maximum yield of hepatoprotective bioactives as compared to other abiotic elicitors used (Thakur et al. 2012).

For enhancing the secondary metabolites of *Phyllanthus amarus* in immobilized cell cultures, MS medium was supplemented with different growth promoters such as gibberellic acid, coconut water, sugarcane juice and watermelon extract. It was revealed that watermelon extract enhances maximum amount of phyllanthin and hypophyllanthin followed by sugarcane juice, coconut water and gibberellic acid (Thakur et al. 2011).

Table: 1 Effect of enhancing materials on the production of secondary metabolites in *Phyllanthus amarus*

S. No	Parts used	Media used	Culture type	Enhancing materials		Result	Reference
1.	Young leaves & flowers	MS, Gamborg B_5 media and White's media	Callus culture	**Phytohormones**		MS media with 2, 4-D and Kinetin) showed maximum percentage induction of callus and also maximum amount of phyllanthin	(Prashanth Kumar et al. 2012)
				2,4-D	1.0 mg/l		
				Kinetin	0.5 mg/l		
2.	Seeds	MS	Suspension culture	**Precursors**		Suspension cultures yield 2 newer lignan 1.Cubebin dimethyl ether and 2.Urinatetralin and production enhanced due to precursors.	(Elfami et al. 2006)
				Ferulic acid	0.5 Mm		
				Caffeic acid	0.5 Mm		

3.	Fresh leaves	MS	Immobilized cell culture	**Phytohormones & precursors**	Cinnamic acid (8ml) treatment gave maximum hepatoprotective bioactive yield	(Thakur and Kharya 2011)
				Kinetin 5,10,20 ml NAA 5,10,20 ml Chitosan 5,10,20 ml Cinnamic acid 2,4,6,8 ml		
4.	Fresh leaves	MS	Immobilized cell culture	**Abiotic elicitors** Chitosan 5,10,20 ml Copper sulphate 2,4,6,8 ml Phenyl alanine 2,4,6,8 ml Silver nitrate 2,4,6,8 ml	Silver nitrate (8 ml) gave maximum phyllanthin and hypophyllanthin content.	(Thakur et al. 2012)
5.	Fresh leaves	MS	Immobilized cell culture	**Growth promoters** Gibberellic acid 30,60,90 ml Coconut water 30,60,90 ml Sugarcane juice 30,60,90 ml Watermelon extract 30,60,90 ml	90ml watermelon extract had highest percentage enhancement of phyllanthin and hypophyllanthin	(Thakur et al. 2011)

VI. CONCLUSION:

The present study describes the immobilized cell culture, callus culture and suspension culture of *Phyllanthus amarus* enhances secondary metabolites by media supplemented with different phyto hormones, growth promotors, precursors and abiotic elicitors. In future, the secondary metabolites of *Phyllanthus amarus* are greatly in demand. So its production can be increased by using micro propagation.

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