Chapter – I

Introduction

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1. Low cost Plant tissue culture

Tissue culture refers to a set of techniques that permit the regeneration of cells, tissues and organs using nutrient solution in aseptic and controlled environment (Lima *et al.*, 2012). Micropropagation or tissue culture method of propagation is one of the techniques with the potential of producing bulk of healthy planting materials without season limitation throughout the year. The recent studies done have shown that a single shoot tip has the potential of producing more than 6000 transformable plantlets per year (Amoo *et al.,* 2011). Tissue culture based plant propagation is carried out in highly sophisticated facilities which are expensive and often are not available in the developing countries. For example the cost media, chemicals, equipment and instruments used in micropropagation such as autoclaves for sterilization of media and instruments are often very expensive. Hence, options to expensive inputs and infrastructure are described and can be sought and developed to reduce the cost in micro propagation (Gitonga, *et al.*, 2010). This necessitates the need to source for alternative low cost equipment and chemical facility studies have addressed the problem by decreasing the unit cost of production like low technology tissue culture materials for initiation and multiplication (Gitonga *et al.,* 2010).

In low cost technology is achieved by improving process efficiency and better utilization of resources. Low-cost tissue-culture technology will stay a high priority in agriculture, horticulture, forestry, floriculture and medicinal plant of many developing countries for the production of suitably priced high quality planting material. In many developed countries, conventional tissue culture-based plant propagation is carried out in highly sophisticated facilities that may incorporate stainless steel surfaces, sterile airflow rooms, and expensive autoclaves for sterilization of media and instruments, and equally expensive glasshouses with automated control of humidity, temperature and day-length to harden and grow plants.

1.1. Economic importance

The potential of plant tissue culture in increasing medicinal plant production and generating rural employment is well recognized by both investors and policy makers in developing countries. However, in many developing countries, the establishment cost of facilities and unit production cost of micro propagated plants is high, and often the return on investment is not in proportion to the potential economic advantages of the technology (Prakash, 1993).

Low cost options should lower the cost of production without compromising the quality of the micro propagules and plants. The primary application of micro propagation has been to produce high quality planting material, which in turn leads to increased productivity in medicinal plant. The

generated plants must be vigorous and capable of being successfully transplanted in the field, and must have high field survival.

1.2. Indian scenario

Low-cost tissue culture technology is the adoption of practices and use of equipment to reduce the unit cost of micro propagule and plant production. Many such facilities established at a high cost are high-energy users, and are run like a super-clean hospital. The requirements to establish and operate such tissue culture facilities are expensive. In India only very low few tissue culture companies are produce tissue culture raised plantlets compare to the developed countries. If the low cost tissue culture technology developed its very useful to the farmers, nursery owners and self-help groups.

1.3. World scenario

Micro propagation has been identified as a suitable technology in the development projects of UNESCO in Africa and the Caribbean; however, the cost of production must be reduced (Brink *et al*., 1998). The private industry is the most important group that requires cost-effective technology. Many international organizations also agree that tissue culture technology is very relevant to agriculture, provided the problem of high cost of production is satisfactorily solved (FAO, 1993). The FAO Committee on Agriculture has perceived plant tissue culture as a main technology for the developing countries for the production of disease-free, high-quality planting material.

1.4. *Tylophora indica* (Burm.f) Merrill

In present study low cost tissue culture studies carried out in *Tylophora indica* (Burm.f) Merrill. (Asclepiadaceae) is an important Indian medicinal plant (Fig- 1). It is called “Asthma kodi” or “Nanjaruppan” in Tamil in the Siddha system of Indian medicine. It is a perennial, small, slender, much branched pubescent twining or climbing herbs or under shrubs. Roots Long fleshy with longitudinally fissured light brown, corky bark. Flowers and fruits are produced between August-December (Kirtikar and Basu, 1975; Chopra *et al*., 1956). The plant has been traditionally used for the treatment of Jaundice, inflammation, antitumor, immunomodulatory, antioxidant, antiasthmatic, smooth muscle relaxant, antihistaminic, hypotensive and antirheumatic activities are scientifically proven.

1.4.1. Propagation in nature

Propagation in nature is only through seeds which are largely limited by low percentage of seed set and seasonal dormancy.

1.4.2. Phytochemicals

In vitro plant tissue culture techniques are not only useful for mass multiplication of rare and endangered medicinal and economically important plants, but also useful for the production of useful bioactive compounds. Plant-produced secondary compounds have been incorporated into a wide range of

commercial and industrial applications, and in many cases, rigorously controlled plant *in vitro* cultures can generate valuable natural products. Plants and plant cell cultures have served as resources for flavors, aromas and fragrances, biobased fuels and plastics, enzymes, preservatives, cosmetics (cosmeceuticals), natural pigments, and bioactive compounds (Benjamin and Mulchandani, 1976).

Tylophora indica are attributed to number of alkaloids (secondary metabolites) present in the plant. Tylophorine is the chief active alkaloid and Tylophorinidine, Tylophornine, Septidine, Antofine and Ficuseptine - C are other important alkaloids encountered. The most remarkable property of *Tylophora indica* alkaloids is in the discovery of these as cytotoxic agents which are equally effective against drug sensitive and multidrug-resistant human cancer cell lines (Bera and Roy, 1993). Tylophorine and its analogs are phenanthroindolizidine alkaloids isolated from *Tylophora indica* and are known to have anticancer, anti-inflammatory, anti-amoebicidal and anti-viral activity (Saraswati *et al.*, 2013).

The secondary metabolites are released due to defense responses which are triggered and activated by elicitors, the signal compound of plant defense responses. Elicitors are compounds which stimulating any type of physiological abnormality of plant. This broader definition of elicitors includes both substances of pathogen origin (exogenous elicitors) and compounds released from plants by the action of the pathogen (endogenous elicitors).

1.4.3. Need for Conservation

Due to its great pharmaceutical importance and demand, the species is rapidly declining at an alarming rate. Unsustainable and indiscriminate collection of the plant from its wild habitat has listed it in vulnerable to extinction category in India (Faisal *et al.*, 2005). Moreover, lack of strategic conservation efforts and poor seed set are constantly threatening the status of this species in future (Thomas and Philip, 2005). Such a situation requires the standardization of efficient propagation methods for sustainable utilization and management of this endangered species.

Despite recent advances in biotechnology, combinatorial biochemistry, and high throughput screening, natural products from plants continue to serve as a major source of new chemical entities for pharmaceutical research. Although these new biotechnological approaches have allowed the creation of new and, in part, semi natural products, the new innovative discipline of metabolomics will clearly open the door to explore thousands of plants and their constituents. Recently developed analytical techniques are capable of identifying and structurally elucidating major constituents in a single plant, or assessing a single biological activity in *in-vitro* assays in parallel.

1.5. Objectives

- To establish a protocol for *in vitro* low cost plant tissue culture.
- To develop a low-cost tissue culture medium using commercially available resource.

- To establish a reproducible protocols for organogenesis from shoot tips and node explants of *Tylophora indica*.
- To develop a suitable low cost tissue culture for high frequency callus production.
- To isolate and characterization of phytochemical analysis from callus using GC-MS.