**Intoduction**

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micropropagation. Different techniques in [plant tissue](http://en.wikipedia.org/wiki/Plant_tissue) culture may offer certain advantages over traditional methods of propagation, including:

* The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.
* To quickly produce mature plants.
* The production of multiples of plants in the absence of [seeds](http://en.wikipedia.org/wiki/Seed) or necessary [pollinators](http://en.wikipedia.org/wiki/Pollination) to produce [seeds](http://en.wikipedia.org/wiki/Seed).
* The regeneration of whole plants from plant cells that have been [genetically modified](http://en.wikipedia.org/wiki/Genetic_engineering).
* The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.
* The production of plants from seeds that otherwise have very low chances of [germinating](http://en.wikipedia.org/wiki/Germinating) and growing, i.e.: [orchids](http://en.wikipedia.org/wiki/Orchids) and [nepenthes](http://en.wikipedia.org/wiki/Nepenthes).
* To clean particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for [horticulture](http://en.wikipedia.org/wiki/Horticulture) and agriculture.

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant ([totipotency](http://en.wikipedia.org/wiki/Totipotency%22%20%5Co%20%22Totipotency)). Single cells, plant cells without cell walls ([protoplasts](http://en.wikipedia.org/wiki/Protoplast)), pieces of leaves, or (less commonly) roots can often be used to generate a new plant on culture media given the required nutrients and [plant hormones](http://en.wikipedia.org/wiki/Plant_hormones).

**Principles and botanical basis of Plant Tissue Culture**

In principle any plant can be propagated using two main types:

**\* Sexually**- by seeds

\* **Asexually- (**vegetatively, also called cloning); by cuttings, division, layering, grafting, etc.

In some ornamental woody plants and under certain conditions, both types may be very difficult or even impossible.

**Sexual** **Propagation** is excluded in the following cases:

* No seeds are formed
* Very few seeds are formed
* Seeds quickly loose their germination viability

**Asexual (Vegetative) propagation** is very important in plant breeding programs, since parent lines need to be maintained and propagate vegetatively for seed production. However, these classical methods may be unsatisfactory in the following cases:

* With plants whose natural rate of increase is too slow
* With new cultivars that is too difficult or can not readily or economically be clonally propagated by these classical methods.
* When it is too expensive to commercially use these methods.

           The field of plant biotechnology provides techniques for rapid propagation of ornamental plants. Many of ornamental plants and woody species are propagated using tissue culture techniques (also known as micropropagation). However, these methods may be difficult to apply to woody plants, particularly in the adult form and most of the reports were dealing with the juvenile form.

           The term micropropagation is used to refer to the application of plant tissue culture techniques to clone (propagate) species using small piece of the mother plant such as shoot tips, meristem tips, lateral buds, etc. to be grown aseptically in a test tube or any other glassware container.

**Organogenesis,**  in embryology, the series of organized integrated processes that transforms an amorphous mass of cells into a complete organ in the developing embryo. The cells of an organ-forming region undergo differential development and movement to form an organ primodium, or anlage. Organogenesis continues until the definitive characteristics of the organ are achieved. Concurrent with this process is histogenesis; the result of both processes is a structurally and functionally complete organ. The accomplishment of organogenesis ends the period during which the developing organism is called an embryo and begins the period in which the organism is called a fetus.

***In vitro* culture of plants can be done via following means:-**

1. Culture of whole plant,
2. Seed culture,
3. Embryo culture,
4. Organ culture,
5. Tissue culture,
6. Single cell culture,

 G. Protoplast culture

## Plant cell culture media

Excised plant tissue and organ will only grow invitro on a suitable artificially prepared nutrient medium which is known as culture medium. Composition of a culture medium has often been modified to stimulate the growth of a particular plant material. Culture media used for the cultivation of plant cells in vitro are composed of three basic components:

1. Essential elements, or mineral ions, supplied as a complex mixture of salts;
2. An organic supplement supplying vitamins and/or amino acids; and
3. A source of ﬁxed carbon; usually supplied as the sugar sucrose.

**MEDIA CONSTITUENTS**

1. **Inorganic nutrients**
2. **Organic nutrients**
3. **Growth hormones**
4. **Gelling agents**

**Macroelement**

As the name, element required by plant in concentration greater than 0.5mMol/litre are referred to as macronutrients. Nitrogen, phosphorus, potassium, magnesium, calcium, and sulphur (and carbon, which is added separately) are usually regarded as macro elements. These elements usually comprise at least 0.1% of the dry weight of plants.

**Microelement**

Elements which required in concentration less than 0.5mMol/litre are referred as micronutrients or microelements. Microelements are essentials as catalyst for many biochemical reactions. Manganese, iodine, copper, cobalt, boron, molybdenum, iron, and zinc usually comprise the microelements, although other elements such as nickel and aluminium are found frequently in some formulations. Iron is usually added as iron sulphate, although iron citrate can also be used. Ethylenediaminetetraacetic acid (EDTA) is usually used in conjunction with iron sulphate. The EDTA complexes with iron allow the slow and continuous release of iron into the medium.

**Organic nutrients**

Mainly vitamins and amino acids are used as organic supplement. Amino acids provide a source of reduced nitrogen and, like ammonium ions uptake causes acidification of the medium. Casein hydrolysate can be used as a relatively cheap source of a mix of amino acids.

**Carbon source**

Sucrose

**Gelling agent**

Media for plant cell culture *in vitro* can be used in either liquid or ‘solid’ forms, depending on the type of culture being grown. For any culture types that require the plant cells or tissues to be grown on the surface of the medium, it must be solidified. Agar, produced from seaweed, is the most common type of gelling agent, and is ideal for routine applications. Purified agar or agarose can be used, as can a variety of gellan gums.

Properties of gelling agent—

1. It should withstand sterilization by autoclaving.

2. Medium should be liquid when hot and form a semisolid gel when cool.

3. It must adequate amount of water to the cell.

**Growth regulators**

PGR are the most important media components which determine the developmental pathway of the plant cells. The PGR used most commonly are plant hormones or their synthetic analogues.

There are five major classes of PGR, namely:

1. Auxin
2. Cytokinin
3. Gibberellins
4. Abscisic acid
5. Ethylene

The most commonly used classes of PGR are Auxin and Cytokinin.

**Auxins**

 It induces cell division, cell elongation, and formation of callus in culture. It promotes root formation when used in low concentrations but when it is used in high concentrations promotes callus formation. The most important naturally occurring auxin is IAA (Indol-3-acetic acid), but its use in plant tissue culture media is limited because it is prone to both heat and light. The commercial analogues are frequently used. 2, 4- Dichlorophenoxyacetic acid (2, 4-D) is most commonly used auxin like compound and is extremely effective in most circumstances.



 Fig. 1 – Auxin (IAA)

**Cytokinins**

It promotes cell division. Naturally occurring cytokinins are large group of structurally related (purine derivatives) compounds. Among naturally occurring zeatin and 2iP (2-isopentyl adenine) have some use in plant tissue culture media. The synthetic analogues kinetin and BAP (6-benzylaminopurin) are more frequently used. The non purine based chemicals such as substituted phenylureas are used as a cytokinin. Sometime phenylureas are used as auxin in some culture system.



 Fig. 2 Cytokinin (Zeatin)

**Gibberellins**

It stimulate cell division and elongation, break seed dormancy, and seed germination. The seeds of some species are tuff to be germinated is soaked on the GA to get them started.

 

 Fig. 3 – Gibberelins (GA3)

**Ethylene**

Its uniqueness is the existing in gaseous state. It induces ripening, cause senescence of leaves. Plant often increases ethylene production in response to stress and ethylene often found in high concentration within cell at the end of plant life. The increased ethylene in leaf tissue in the fall is part of the reason leaves fall off tree.



 Fig. 4 – Ethylene

**Abscisic acid**

It is a general plant growth inhibitor. It induces dormancy and prevents seeds from germination, causes abscission of leaves, fruit, and flowers and cause stomata to close.

 

 Fig. 5 – Abscisic acid (ABA)

**Brief introduction of the experimental plant ( *Tectona grandis*)**

*Tectona grandis* is a premium quality timber species internationally known as "Teak". The tree is commonly known as sagwan in India.

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| **Botany** *Tectona grandis* is predominantly a single-stem deciduous tree with a dome shaped crown of lush green foliage. On wet sites it may remain evergreen. The trees reach a height of 20-40 meters with a girth of 1.5 - 2.0 meters (Prasad *et al.*, 1993). Leaves are alternate, odd-pinnate with 5-7 unequal-sized leaflets originating from the same rachis. Leaflets are broadly obtuse, dark green above and pale below. Flowers are white in axillary panicles, 0.5-1.0 cm long.  The brown pods are oblong-lanceolate and pointed at both ends. They contain 1-4 smooth brown seeds and do not open at maturity. The bark is grey, thin with irregular short cracks, exfoliating in fibrous longitudinal flakes (Troup, 1921; Kadambi, 1954). The root system is well developed, consisting of deep tap roots and long lateral roots. When near the soil surface, roots produce suckers.**History of cultivation**It was introduced into some countries in Asia, including Sri Lanka where there are a few trees under natural conditions some in the Peradeniya Botanic Garden. Plantations were started in 1873 in Java. The species has also been introduced into African countries, including Nigeria, Tanzania and Kenya.**Natural Habitat**it is reported to be scattered in the dry deciduous forests throughout the Indian pinsula. It grows in the sub-Himalayan tract from Oudh eastwards to Sikkim, Bihar, Orissa, and throughout central and southern India. Optimum growing conditions for the species are reported to be in the Bombay region.**Geographic Distribution**Native : India, IndonesiaExotic: Kenya, Malaysia, Myanmar, Nepal, Nigeria, Philippines, Sri Lanka,Vietnam**Biophysical limit**Altitude: 0-1 500 m, Mean annual temperature: 8-44o C, Mean annual rainfall: 750-5 000 mm **Soil type** The species grows on a variety of soil formations including gneiss, trap, laterite, alluvial, and bolder deposits. It grows best on well-drained, deep, moist soils. it is common on deep loams or clays containing lime. It also grows well on black cotton soils. Shallow dry soils and poor drainage stunt tree growth.**Reproductive Biology**In drier natural habitats, its sometimes sheds leaves (either partially or sometimes fully) by the end of January and fresh foliage appears in April-May. Flowering begins by December and normally continues to March or rarely October. In moist conditions, the trees remain evergreen throughout the year. The species in the genus Dalbergia have developed wings and hairs and often air spaces within the seed coat or pericarp. They are therefore normally wind-dispersed.**Uses****Wood:-** The sapwood of *teak* is pale yellowish-white often with a tinge of purple. Heartwood varies in color from light golden brown to shades of light purple with dark streaks, or deep purple with distant black lines. The heartwood darkens with age and weighs about 850 kg per cubic meter. The wood is very hard with no distinct annual rings. It is difficult to work because of its high density. The wood is fragrant and commands a high price. It is used to make premium-grade furniture, paneling, veneers, and interior and exterior joinery. Secondary uses of the wood include; knife handles, musical instrument calico-printing blocks, mathematical instruments, agricultural implements, and boats keels and screws.

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|  **Medicinal uses** Tannins from the bark are used to produce medicines for the treatment of diarrhoea, worms, indigestion, and leprosy. These tannins also produce an appetizer.**Conventional methods of propagation**Under natural conditions, its reproduces by seed, root sucker or coppice. Artificial reproduction is common by seed, root cutting, and stump sprout. Direct seeding is possible under moist conditions with good weed control. Root cuttings can be planted directly in the field or raised in a nursery for future transplanting. Nursery grown seedlings are transplanted to the field after six months in Java, and 12 months in India. Although no treatment is necessary, soaking seed in cool water for 12-24 hours hastens germination.**Necessity for non conventional propagation methods** The indiscriminate cutting of these trees from their natural habitat to meet the high market demand has resulted in a significant decline in the population of this species.  Conventional propagation of this species is only through seeds; however, its success is limited due to poor seed viability (Raghava Swamy *et al*., 1992). Thus, there is a need for developing a method for large scale propagation. Efficient *in vitro* propagation could be a reliable method for the establishment of new bamboo plantations and they offer an attractive alternative to conventional methods for mass propagation. Micropropagation is being widely used for producing large numbers of propagating material in different tree and crop plants within a limited time. |

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