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In vitro propagation and phytochemical studies of Indian Teak (*Tectona grandis* L.)

R. Sreedevi and. T. Damodharam*

Department of Environmental Sciences, S. V. University, Tirupati

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ABSTRACT

Multiple shoot formation was induced from excised terminal buds of hundred years old elite tree of Tectona grandis(L.) on a defined medium supplemented with various concentrations of 6-benzyl amino purine and kinetin either singly or in various combinations. The highest shoot regeneration frequency (100 %) was obtained from nodal segments on MS medium fortified with 2 mg/l IBA and 1.5mg/l IAA. The regenerated shoots best on MS 2mg/l IBA. Regenerated plantlets with well developed shoot and roots were hardened, successfully transferred to soil and maintained in green house. Further the work continued confer about qualitative phytochemical tests conduct to the presence of some of the secondary metabolites of teak leaves. Result revealed the presence of phenols, flavonoids, tannins, Proteins, and Aminoacids.

Key words: MS medium, Phytochemicals, Secondary metabolites, Terminal buds, *Tectona grandis*

INTRODUCTION

Tectona grandis L., (Verbenaceae) is the major tropical hard wood plantation species is a useful indicator for plantation trends in 1980. It constituted 11% of the total area of tropical forests plantation where as in 2010 the corresponding figure was 4%. If the present trend persist, the future outlook for the sustainable supply of quality tropical hardwood is miserable. This is at a time when demand for the raw material, particularly domestic demand , is a forecast to rise, what is required now, to avoid a pending crisis in the sustainable supply of tropical hardwoods is to increase , sustainably, the area under sustainable management in natural forests and supplement this with a significant increase in plantations.

Teak, one of the best-known tropical timbers, is native to the Indian subcontinent extending to adjoining Myanmar, Thailand and Laos teak forests of peninsular India are classified into moist, semi moist and dry categories depending on the quantity of rainfall received [1] . Genetic differences are known to occur between varies provenances [2] tested within and outside natural geographic range of the species and provenances from western India are reported to be 30% faster growing than the local seed source in Ghana [3]. In addition, tropical tree species are valuable as ornamentals for landscaping (shade, flower, fall foliage and fruit production) and as a source for a multitude of known and yet unknown commercial properties, such as medicinal drugs, natural insecticides, industrial uses and non timber products. *Teak* also holds the medicinal values; the bark is bitter tonic and is considered use full in curing fever. It is also use full to cure head ache and stomach problems, digestion may be enhanced by the teak wood or bark. Therapeutic uses lie cups eye diseases and vomiting. *Teak* tree grown in moist and deciduous forests contain oil that has strong and distinctive scent. The juice that obtained from the leaves of the tree can be used as fabric dye as well the paste prepared from powdered wood of *teak* tree is considered astringent diuretic, hepatic stimulant, sedative and a local refrigerant. It is further recommended for reducing inflammatory swelling and toothache.

Poor seed production and seed germination has a major limiting factor in organizing open-pollinated breeding populations [4] of natural trees. Teak improvement in India has focused on grafting plus trees from natural forests and establishing clonal seed orchards. Tropical hard wood tree species are significant economically and ecologically and play a major role in the biodiversity of plant and animal species with in an ecosystem. Many of these tree species are being threatened and are endangered because of logging practices, conversion to agricultural lands, on optimal management strategies, and overall deforestation rates that cannot keep up with natural regeneration of native forests. Tropical tree species provide timber for commercial uses because of the beauty of the wood grain, color, pattern, strength, durability and versatility of finishing applications for a vast array of markets. Because of the high value of tropical tree species, tissue culture (micro propagation) is a proven means of producing millions of identical plants by culturing plant tissues under germ-free-conditions. Micro propagation provides a high degree of phenotypic physical uniformity. Since the product on cycle takes place under controlled conditions, the resulting product has very high degree of uniformity compared with traditionally propagated plants. Plantlets produced by tissue culture are usually disease free. With reference to an integral component in tree improvement and conservation programs, in order to complement seed banking and ex-situ measures for long term conservation and clonal propagation of tropical tree species are also important because wild life population may be affected, soils can be stabilized and organic matter and nutrients in the forest floor altered, degraded areas can be restored and tropical trees also provide socio-economic development for local communities. In vitro propagation has been successfully applied to teak and became an alternative tool to overcome some problems occurring in sexual regeneration. Currently, mass propagation of selected teak clones is possible through in vitro multiple shoot production .Regenerative organs such as pre-existing shoots, meri stem shoot tips, nodal segments or seedling organs have been widely used as explants [5 6 7 8] . The development of invitro regeneration procedure is required not only for the propagation of superior genotypes, but also for the regeneration of genetic improved plants. The present investigation focuses on invitro adventurous shoot regeneration propagation of tropical tree species grown or harvested for timber for the development of an invitro regeneration procedure for Teak improvement from inter nodal segments. Benefits of tissue culture teak plants will grow straight and maintain uniformity. The growth of all plants will be equal which gives expected yield and also more resistant power to pests and diseases.

MATERIALS AND METHODS

Mature nodal segments (NS) of the naturally grown healthy plant of *Tectona grandis* L. were used as the source of explants material for the present study. The explants were collected, washed thoroughly under running tap then treated with 10% teepol (qualigen fine chemicals , Mumbai India) for 20 min, washed three times with sterile Millipore water. Finally, the explants were dipped in 70% ethanol for 30 sec followed by rinsing with Millipore water after rinsing four times with Millipore water, explants were treated with 0.1 % mercuric chloride solution for 3 min and washed thoroughly in sterile Millipore water. After sterilization the explants were placed on tissue paper for removal of moisture on the surface and then the nodal segments were trimmed at both the ends to appropriate size (1-1.5 cm) and cultured on sterile media. Murashige and Skoogs medium [9] for plantlet development different organic additives were added to the basal medium to determine the factors affecting the morphogenic response at different stages of the culturing process. Full strength ms medium used as basal medium. It was supplimented with various plant growth regulators like indole -3-buteric acid (IBA), indole -3-acetic acid (IAA) in different concentrations and combinations .The PH of the medium was adjusted to 5.8 before autoclaving. All inoculations were carried out in a laminar air flow cabinet cultures then inoculated in a culture room at 25^{o} C and a 16 hrs light 8 hrs dark period, observations were taken at regular interval for growth and contamination , all cultures were renewed by sub culturing every 4 weeks and the contaminated cultures were discarded. The explants derived shoots were transferred to MS medium supplemented with different concentrations of cytokines in combination with auxin (Kin+NAA) using the conditions described for shoot induction ,40 shoots were cultured and is the number and length of shoots were determined after 30 days of culture the experiments were repeated in triplicate

Biochemical analysis:

Fresh leaves were collected from mother plant for various biochemical analyses, in which estimation of carbohydrates was performed by Sadasivam and Manickam et al (1992). Proteins by Lowry et al (1951), alkaloids were determined by Harborne, 1973, Flavoind by Boham and kocipai-Abyazan et al1974, terpenoids Morigiwa 1986, estimation of phenols Thimmaiah, 1999 and tannins were performed by using[11.] Sadasivam and Manickam et al (1992).

Acclimatization:

Plants multiplied invitro are exposed to an optimal growth condition which may support rapid growth and rendering them unfit for survival under in vivo plantlets with well developed shoot system were removed from culture medium. Excise the medium part from shoots by using sterile blades and were placed in NAA(Napthalene acetic acid) for few minutes then acclimated using plastic pots containing sterile vermiculite in the incubation chamber.

The plastic pots were covered with holed polythene bags and regularly supplied with half strength MS basal liquid medium. The plantlets were hardened using pots containing vermiculite and soil mixture (1:1) under green house condition and after 4 weeks they were transferred on to earthen pots containing garden soil, sand and cow dung (2:1:1) for hardening and acclimatization[10] and maintained under normal day light condition thus it is possible to develop an efficient invitro regeneration and mass multiplication of plants.

RESULT AND DISCUSSION

A protocol for rapid propagation of *Tectona grandis* (L) through nodal explants had been established. Nodal explants were cultured on MS medium containing various concentrations and combinations of cytokines 6-Benzyl aminopurine, Indo 3- acetic acid, Indole 3-butyric acid (BAP, IAA and IBA). The shoot regeneration efficiency were tabulated in (Table I). From these result it was understood that the BAP (2.0 mg/l) and IAA(1.5 mg/l) showed better response 5.5±0.20 then others. The shoot elongation was observed in BAP (2 mg/l) show better response. The multiple shoot induction of the regenerated micro shoots were found to be the combination of BAP (2mg/l) and IBA (1.5 mg/l) as shown in Table 1 which showed 100% response. Approximately 80% of the node explants remained aseptic three to four days after incubation on establish medium, as shown in figure 1,it was observed that The auxiliary buds started to burst and one auxiliary shoot developed per node (Figure II (ii), rarely two, however only one was able to elongate. The process of teak culture establishment in this experiment coincided with[6] , who reported that auxiliary shoots developed after 7-10 days and with auxiliary shoots after 6 weeks on several nodes.

In the present study rapid micro propagation was achieved through induction of multiple shoots from auxiliary bud explants. A rapid rate of propagation depends on the sub culturing of proliferating shoot cultures. In the case of prolonged cultures the nutrients in the medium gradually depleted[17] . reported propagation profile for picrorhiza kurroa and observed that the shoot multiplication rate gradually improved as the number of subcultures increased. The shoot multiplication is enhanced by subsequent cultures the observations in this study are in agreement with reports such as gymnema sylvestre[18], Hemidesmus indicus[19] and holostemma adakodien [20]. Subculturing within 4 weeks was essential to maintain healthy shoot growth, moreover, repeated subculture of the original medium produced a crop of shoots

Figure I: Plant regeneration through nodal segments (NS) of *Tectona grandis* L.f., (i). Induction of buds opening on MS+ growth hormones from nodal segments after one week of culture (ii). Multiplication and proliferation of shoots on the same medium after 3 and 6 weeks, respectively (iii, iv and v). Multiple shoots from MS+ growth hormones (vi) Rooting on vermicompost at shade house

i: bud open

ii: Auxiliary shoot Development

iii: Multiplication and proliferation stage 1

iv: Multiplication and proliferation stage 2

v: Multiplication and proliferation stage 3

vi : Rooting on vermicompost

Table I: Effect of plant growth regulators on shoot initiation of *Tectona grandis L.*. in MS medium after 6 weeks of culture

Plant growth regulators mg/l			%Regeneration per explants	No. of shoots per explants	Shoot length
BAP	IAA	IBA			
1.0			40	2.8 ± 0.11	9.9 ± 0.15
1.5			50	4.9 ± 0.15	9 ± 0.57
2.0			70	7.2 ± 0.15	8 ± 0.29
2.5			60	8.8 ± 0.24	5 ± 0.08
2			60	2.6 ± 0.11	10.4 ± 0.15
2	1.0		100	5.5 ± 0.20	9.7 ± 0.31
2	1.5		50	7.5 ± 0.11	9.7 ± 0.5
2	2.0		80	8.6 ± 0.17	7.2 ± 0.23
2	2.5	1.0	70	6.9 ± 0.05	10.1 ± 0.17
2		1.5	100	7.4 ± 0.11	9.7 ± 0.15
2		2.0	60	6.5 ± 0.05	8.9 ± 0.15
2		2.5	80	6.2 ± 0.17	6.7 ± 0.14

Values represent means ±standard error of 3 replica tes per treatment

Phytochemicals analysis:

Plant cells produce a vast amount of secondary products. Many of these are highly toxic and are often stored in specific vesicles or in the vacuole. Several studies indicate that this kind of storage functions on one hand as a detoxification of the plant itself and generates on the other hand a reservoir of, for example, nitrogen-rich molecules. In contrast to animals, these are not excreted by plants. Some secondary plant products can be reversibly degraded and are fed into the basic metabolism while others cannot. The preliminary phytochemicals analysis of *Tectona grandis* leaves were carried out using different solvent extractions.

Plants produce high diversity secondary metabolites for defense and survival in the ecosystem. Medicinal herbs practiced in traditional floke medicine in India were screened for the treatment of many diseases added back to pre history and people of all continents have this old tradition. Plants have long been and continue to be the basis of many traditional medicines worldwide. Asian traditional medicinal systems such as traditional Chinese medicine (TCM), Korean Chinese medicine, Japanese Chinese medicine (Kampo), Ayurveda from India and Jammu from Indonesia are well known [8]. Phytochemicals are the compounds derived from the plants among which most of them are found to possess several medicinal attributes, though they are non nutritive. Further detection of the presence of phytoconstituents from plants will help the pharmaceutical industry to save time and cost. Phytochemicals have many ecological and physiological roles as widely distributed plant constituents. Phytochemicals exhibits wide range of biological effects on constituents with their own antioxidant properties. The bio active compounds of Tectona grandis was listed in Table. ii. Phytochemicals analysis of the extract indicated the presence of 8 compounds. The leaves showed a number of phytoconstituents in methanol extract. The alkaloids have strong anticancer properties. Tannins are naturally occurring, water soluble phenolic compounds. The primary source of tannins used as an active pharmaceutical agent are from plants [11]. implying that the pharmacologic effect of tannins depends upon the plant type. Tannins have been found to form irreversible complexes with highly rich protein resulting in the inhibition of cell protein synthesis [20] they are known to react with protein to provide difficult tanning effect which is important for the treatment of influenced or ulcerated tissues. Herbs that have tannins as main component have astringent activity and are also used for the treated intestinal disorder such as diarrhea and dysentry.Tannins in mimosapudica is exploited in the traditional treatment for ailments [21].

Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [22]. Natural antioxidants mainly comes from plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols etc [23]. Flavonoids are the most important group of secondary metabolites and bioactive compounds in plants [24]. Flavonoids are also known as vitamin P or citrine. These metabolites are mostly used in plants to produce yellow and other pigments which play a big role in coloring the plants. Flavonoids possess adiverse biological activities,

antidiabetic activity and anticancer activity have been reviewed [25]. Flavonoids are also closely related to flavones which are actually a sub class of flavonoids and are yellow pigments in plants

Table. II: Phytochemicals screening of *Tectona grandis* (L.f)

Bioactive Compounds	Ethyl acetate	Chloroform	Ethanol extract	Methanol extract	Water
Alkaloid	--	--	--	--	--
Amino acids	++	+	++	++	++
Carbohydrates	++	++	++	++	++
Flavonoids	--	--	++	++	--
Phenols	--	--	++	++	--
Proteins	++	++	++	++	++
Tannins	--	--	++	++	--
Saponins	++	++	++	++	++
Steroids	++	++	--	--	++
Cardiacglycosides	++	++	+	++	++

++ = presence, -- = absence

Carbohydrates are one of the most important components in many foods. Carbohydrates or saccharides are sugars and starches, which provide energy for humans and animals and cellulose which make up many plant structures. Proteins are large biological molecules consisting of one or more chains of amino acids. Proteins perform a vast array of functions within living organisms, including catalyzing metabolic reactions, replicating DNA, responding to stimuli and transporting molecules from one location to another. Terpenoids are the modified or oxidized terpene is called as terpenoids. Terpenoids consists of around 55% of major group of plant secondary metabolites. The antimicrobial activities of terpene are reported[1921]. The application and future potency of terpenoids are reviewed by[26].

Cardiac glycosides are class of natural products that are traditionally used to increase cardiac contractile force in patients with congestive heart failure and cardiac arrhythmias[27]. Therapeutic effect of cardiac glycosides in breast cancer has been known since 1979. The role of cardiac glycosides in cancer research and cancer therapy is reviewed.

CONCLUSION

A protocol for tissue culture and regeneration of plantlets from mature nodal segments of *Tectonagrandis* has been developed. The result also indicate that regeneration is possible with all cultures that requires a higher degree of standardization for improving the regeneration efficiency. The screening of phytochemicals constituents of experimental plant indicated the presence of carbohydrates, flavonoids, tannins, and terpenoids. There is a need for further investigations using fractionated extracts and purified chemical components.

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