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Studies on the Invitro Regenerative Response of *Santalum Album*

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Abstract:
Trees are an integral part of human life, and vital components of biodiversity forest in particular are renewable sources of food, fodder, products. In recent years there is a tremendous reduction in forest vegetation due to the marked increase of population and also the human desire to progress. To protect the areas of forest vegetation, conventional approaches have been employed in the past for propagation and improvement. In recent years due to biotechnological interventions for in vitro regeneration, mass micro propagation and gene transfer methods in forest tree species have been practiced with great success.
Though there exists the limitations of long juvenile phases and lifespan, development of plant regeneration protocols and genetic engineering of tree species are gaining importance in present trends. By Genetic engineering there is a possibility of introducing a desired gene in a single step for precision breeding of forest trees. This study of santalum album attempts to overview the progress in tissue cultural genetic transformation and biotechnological applications in the present and also focuses the future implication.

Keywords: *Santalum album, sandalwood, explants, micropropagation, shoot tip*

1. Introduction

Santalum album is a woody plant belonging to Santalaceae family (Sandalwood family) and it is commonly known as East Indian sandalwood, is among the oldest known perfumery material and is highly acclaimed worldwide.
Santalum album is a small to medium sized evergreen tree, sometimes growing to 20 meters height. It generally grows in open forest. It is a semi parasitic plant that uses nutrients from host species to help it grow. More than 300 plants have been recorded as hosts that include herbs, shrubs, nearby grasses or trees. Trunk-sapwood is white and unscented. Heartwood is strongly aromatic and yellowish in colour [1]. The wood is widely used in carving. Flowers from the age of about 3 years, it flowers twice a year during March - April and September- October. There are about 19 species of Santalum, but sandalwood has the highest oil content. Seed production generally is good in one of the seasons. About 6000 seeds make 1kg. Seeds can be collected directly from the tree. The fruits should be depulped, washed thoroughly in water, dried under shade and stored in air tight containers [1].
The problems in sandalwood seedling are as follows [2]

1. Quality of seed directly depends on conditions of mother trees which should be more than 20 years old, having high santalol.
2. Since it is a semi parasite plant and hence there exists competition between sandalwood and the host plant.
3. High humidity will decrease the aeration condition and unsuitable for sandalwood seedling growth. Therefore, the regulation of moisture plays a vital role in producing high quality seedlings.
4. The type of sowing medium can influence the aeration and drainage capacity and both will influence the survival of seeds.
5. High intensity of light can kill the seeds, therefore shading is required for higher survival rate
6. The leaf worm, the most common pest can quickly kill a large number of seedlings.
7. The knowledge, skill and technology to develop sandalwood plantations is essential for the local communities and official governments.

1.1. Chemical Constituents

Sesquiterpenals; (includes 80 to 90% terpeniod alcohols including *a* and β-santalols (67%), which is a mixture of two primary sesquiterpenic alcohols) santalic and teresantalic acid, aldehyde, pterocarpin and hydrocarbons, isovaleric aldehyde, santene, santenone [3].

1.2. Micro Propagation

Multiplication of genetically identical copies of plant species by a method of asexual reproduction is called "clonal propagation" and plant population derived from a single individual by asexual reproduction constitutes a "clone". In nature, clonal propagation occurs by apomixis (seed development without meiosis and fertilization) and/or vegetative reproduction (regeneration of new plants from vegetative parts). Apomixis being restricted to only few species, the method of vegetative propagation of plants has been practiced for centuries through improved conventional methods. Plant tissue culture has become a popular method for vegetative propagation of plants. Large scale clonal propagation of plants popularly known and described as "micropropagation" is the first major practical application of plant biotechnology.

Morel and Martin (1952) for the first time recovered virus free Dahlia plants from infected individuals by excising and culturing their shoot tips in vitro. Morel (1960) also realized the potential of this method for rapid propagation of these plants. This contribution of Morel not only revolutionized, but also gave impetus to the utilization of shoot-bud culture for rapid cloning of other plant species in the orchid industry [11].

The micropropagation technology is simple involving the culture of isolated shoots/shoot bases/shoot meristems on suitable nutrient media to stimulate the development of multiple axillary buds by overcoming apical dominance. Generally three to six-fold increase in the shoot numbers at regular 4-6 weeks interval results in the production of a large number of plants from each cultured shoot meristem in one year[12]. The simplicity of the methodology has allowed development of hundreds of small and large industries in many countries around the world.

The success of a micropropagation protocol depends on a number of factors. The age, nature, origin and physiological state of the inoculum/explant and seasonal variation play a crucial role in the establishment of cultures and subsequent plant regeneration. Sterilization method, media and culture conditions also affect the micropropagation.

1.3. Explants

Since the objective of micropropagation is large-scale production of genetically uniform planting material, the role of explant becomes very important in determining the success of any micropropagation system. An explant is an important independent living unit capable of presenting all genetic information that the excised cell(s) or tissue(s) perform at their original location. Murashige (1974) recognized several factors that should be considered in explant selection viz., the organ that is to serve as a tissue source (nature and origin of the explant), the physiological and ontogenic age of organ, the season in which the explant is obtained, the size of explant, the overall quality of the mother plant, the position of the explant on the mother plant or stock plant, orientation of the explant on the medium and the inoculation density. Therefore, it is imperative to consider these points before selecting any explant for the micropropagation system because the ability of regeneration of any explant is influenced by above mentioned factors.

1.4. Applications

Sandalwood is believed to be indigenous to the Indian subcontinent. Due to the therapeutic properties of sandalwood, it has found many applications all over the world such as production of perfumes and incense, and treating numerous types of health problems. Today, sandalwood is considered a highly valuable and expensive raw material available in the production of western perfumery.

Sandalwood has a natural deoderizing property. The tantalizing smell of sandalwood and sandalwood oil is legendary. One sandalwood tree in the forest is known to make the whole forest smell of sandalwood. The warm and sweet, yet non-overpowering smell makes it a natural choice for creating a wide range of expensive perfumes. Sandalwood oil is also used in aromatherapy.

Sandalwood oil has a sedative and calming property. As a result, sandalwood oil is found its way in the treatment of insomnia, stress and depression. It is thought to naturally control anger and aggression. Sandalwood also has a property of inducing a meditative state and hence, found in many temples and churches.

Sandalwood oil can act as a natural antiseptic particularly as a pulmonary antiseptic oil. It can also be used in the treatment of urinary tract infections and cystitis. However, care must be taken that sandalwood oil should not be used to treat urinary tract infection without a doctor's guidance.

Its antiseptic properties have been put to good use in healing scars, wounds and acne.

Sandalwood acts both as a powerful astringent (substance that cleans the skin and contracts the pores) and moisturizer. This makes it useful for treating both oily and dry skin as well as dehydrated and chapped skin. It can also be used to treat allergic rashes.

Sandalwood is a powerful digestive agent. Traditionally, sandalwood has found its applications in treating digestive complications arising from nausea, diarrhea, gastritis and colic. It is found as a digestive muscle relaxant.

Sandalwood's anti-inflammatory property helps it to be used in treating skin inflammations. It also helps in treating neuralgia, insect bites, poisoning and wounds. According to Julia Lawless, sandalwood has been used in eastern medicine for ages to treat skin problems, diarrhea and stomach ache.

Sandalwood's expectorant and bactericidal properties are used to treat viral infections such as flu and cold to help relieve sore throats, catarrh and coughs. Traditional Indian Ayurvedic medicine uses sandalwood for respiratory infections [4].

2. Materials and Methods

2.1. Standardization of Explants

2.1.1. Explants

The experiment was performed using single node, inter node, and shoot tip, petiole and leaves of *Santalum album* of less than 5 years old or five year old plant. The explants were inoculated on Murashige and Skoog media (MS, 1962) supplemented with growth factors (6- Benzyl Amino Purine (BAP) and 2, 4 – Dichloro phenol (2, 4 - D). The culture bottles and test tubes were incubated in growth room at 28ºC, with light intensity of 10 lux and light and dark cycles of 8 – 16 hrs to standardize the explants.

2.2. Micropropagation

2.2.1. Media Components

The MS media used for the micro propagation is prepared by addition of the micronutrients, macronutrients and some other components such as vitamins, carbon source etc.

2.2.2. Culture Media

Murashige and Skoog medium (Chawla, 2010) was used for culturing the explants.
The composition of the media is presented in Table.1

Composition	Concentration(mg/l)		Composition	Concentration(mg/l)
Macronutrients			***Micronutrients***	
NH_4NO_3	1650		$MuSO_4.4H_2O$	22.3
KNO_3	1900		$ZnSO_4.7H_2O$	8.6
$CaCl_2.2H_2O$	440		$CuSO_4.5H_2O$	0.025
KH_2PO_4	170		$CoCl_2.6H_2O$	0.025
$MgSO_4.7H_2O$	370		KI	0.83
Vitamins			H_3BO_3	6.2
Thiamine HCl	0.1		$Na_2Mo_4.2H_2O$	0.25
Pyridoxin HCl	0.5		$FeSO_4.7H_2O$	27.8
Myoinositol	100		Na_2EDTA	37.3
Nicotinic acid	0.5			
Aminoacids				
Glycine	2			
Carbon Source	30000			
Gelling agent agar	8000			

Table 1: The composition of the media

2.2.3. Preparation of medium

Full strength MS medium was used as the basal medium in all the cultures. Auxin or cytokinin at the concentration of 1, 3, and 5 mg/L was supplemented to the basal medium. 3% sucrose was added and the pH of the medium was adjusted with 0.1 N NaOH or 0.1 N HCl to 5.7±0.1. The medium was then supplied with 0.8% (w/v) of plain agar powder and autoclaved at 121°C, for 15 minutes. 25 ml medium was poured into culture bottles and covered with aluminium foil prior to autoclaving. After autoclaving, the media were stored in the culture room prior to initiation of treatment.

2.2.4. Explant

The experiment was performed using single node, leaves and shoot tip of *Santalum album* of less than 5years old. The explants were inoculated on Murashige and Skoog media supplemented with growth factors (BAP & 2, 4-D). The culture bottles and test tubes were incubated in growth room at 28ºC, with light intensity of 10 lux and light and dark cycles of 8-16 hrs.

Figure 1(a): Shoot tip explants *Figure 1(b): Leaf explant* *Figure 1(c): Single node explant*

2.2.5. Leaf Inoculation

Young mature light green coloured leaves were collected from the branches of a five year old tree. Leaves of length ranging from 3.5 to 4.5cm were chosen. Before choosing these leaves a study of the growth pattern by Allometry method was done to choose the point of growth. For histological examination, very thin layer of longitudinal section was taken and observe under the microscope and observe the pattern of arrangement of cells. The excise intact leaves were first cut parallel 0.5cm on either side of the mid rib. These strips along with midrib were made into very tiny sections by cutting perpendicular to the mid vein with both the adaxial and abaxial i.e. dorsal and ventral sides of the leaf.

The leaf explants were surface sterilized in the following manner.

10 minutes under running tap water the explants were washed.

Then they are dipped and thoroughly washed in Tween- 20 for 10 minutes. (0.1% v/v)

It is followed by rewash with distilled water for 10 minutes.

The explants are then treated with solution of fungicide (Mancozeb) for 10 mins.

The explants were pre-soaked with anti-oxidants (citric acid)

2.3. Steps followed in Laminar Air Flow Chamber

The explants were washed with alcohol for 30seconds.

Then they are washed with distilled water for 4-5 minutes.

The explants are treated further with $HgCl_2$ (0.01%) for 15 minutes with intermittent washing with distilled water.

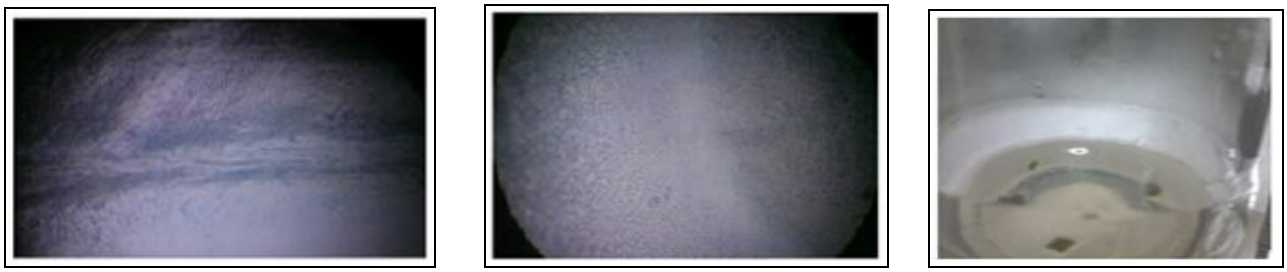

Figure 2(a): Allometeric examination of leaves *Figure 2(b): Magnified view of L.S. of leaves* *Figure 2(c): Inoculated leaf explants*

2.3.1. Single Node Inoculation

Healthy and disease free shoots were collected from the branches of a five year old tree. Young branches were selected and the nodes were collected by excising the branches and the main branch and the nodes along with the buds were separated by reducing the explant size to 0.5cm. Care was taken not to damage the bud. Buds were surface sterilized by the procedure followed for the leaves.

Figure 3: Inoculated single node explants

2.3.2. Shoot Tip Inoculation

Disease free shoot tips were collected from the branches of a five year old tree. Young shoot tips were excised from the branches and tender leaves were separated and inoculated.

The culture bottles were incubated at 28ºC in growth room, with light intensity of 10 lux and light and dark cycles of 8-16 hrs.

Figure 4: Inoculated shoot tip explant

2.3.3. Growth Regulators Used

Two different growth regulators were used i.e. BAP (6-Benzyl-Amino-Purine) and 2, 4-D. the effect of growth regulators was evaluated on the basis of percentage of explant survival. For each of three explants three replicates were carried out.

2.3.4. Shoot Induction From in Vitro Direct Explants

Among the three explants (leaf, shoot tip and single node) only the single node and shoot tip were responded with the growth regulator concentration of 5mg and 4mg of BAP.

S. No.	Growth regulators	Concentration(mg/L)
1.	BAP	1mg/l 3mg/l 5mg/l
2.	2,4-D	3mg/l 5mg/l

Table 2: Growth regulator combination

For all the combination taken, one control was maintained.

2.4. Experimental Analysis

The experiment was conducted on direct organogenesis & callusing with 3 replicates for each treatment and 3 replications for control for each explant.

Data was recorded with respect to time taken for organogenesis & callusing respectively.

S. No	Treatment(explants)	No. of days for sprouting
1.	Single node	10days
2.	Shoot tip	7Days
3.	Leaf	No response

Table.3: No of days for initial sprouting

S. No.	Explants	Growth regulator conc.(mg/l)	No. of days
1.	Single node	BAP1mg/l	-
		BAP3mg/l	-
		BAP5mg/l	10
		2,4-D3mg/l	10
		2,4-D5mg/l	-
2.	Shoot tip	BAP1mg/l	-
		BAP3mg/l	7
		BAP5mg/l	7
		2,4-D3mg/l	7
		2,4-D5mg/l	-
3.	Leaf	BAP1mg/l	-
		BAP3mg/l	-
		BAP5mg/l	-
		2,4-D3mg/l	-
		2,4-D5mg/l	-

Table 4: Explants and growth regulator concentrations

2.5. Observations Recorded

- Contamination studies: The number of fungal and bacterial contaminated culture bottles were observed and recorded then expressed as percentage.
- Survival percentage: The number of explants which remained healthy were recorded and expressed as percentage.
- Percentage of aseptic culture: The number of fungal and bacterial contamination free culture bottles were observed and recorded then expressed as percentage.
- Days taken for shoot initiation: The number of days taken for shoot initiation were recorded and expressed as percentage.
- Studies on effects of growth regulator concentration on shoot initiation: Number of days taken for initial sprout, number of shoots per explants and shoot length was measured and recorded in centimeters.

3. Results

The experiment was conducted to observe which explants are well suited for invitro micro propagation hence standardization of explants was done before invitro propagation.

3.1. Standardization of Explants

For standardization, five explants were taken such as shoot tip, single node; inter node, petiole and leaf.

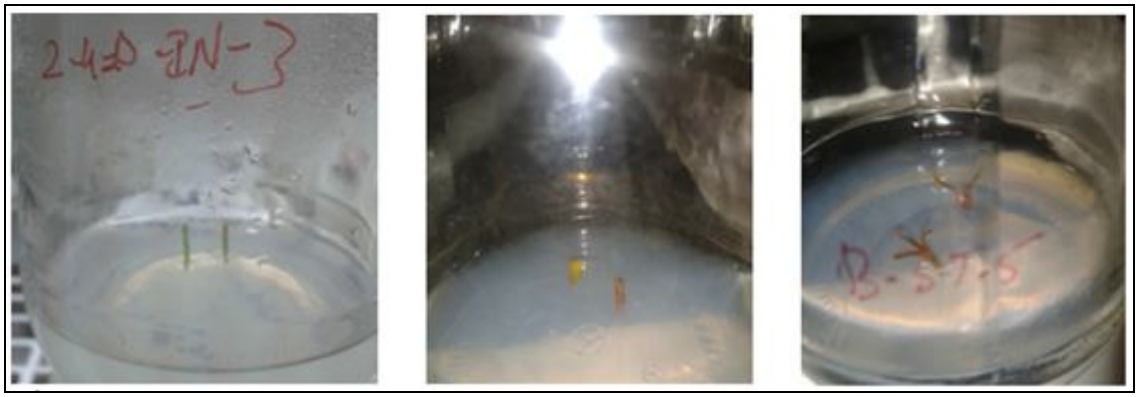

Figure 5(a): Inoculated petiole explants *Figure 5(b): Inoculated inter node explant* *Figure 5(c): browning of shoot*

After few days of inoculation, there was browning of explants and some of them were contaminated.

3.2. Contamination Percentage

Statistically significant differences were noticed among different explants i.e., single node, leaf, inter node, shoot tip and petiole explants for contamination percentage. It was observed that imperfect surface sterilization and low or high concentration of mercuric chloride leads to this contamination.

Figure 6(a): Contaminated single node explants *Figure 6(b): Contaminated leaf explants*

3.3. Survival Percentage

The survival percentage of different explants was observed and the survival percentages of the explants were different.

The survival percentage of shoot tip was recorded as (80%), single node as (40%), leaf as (20%) and petiole and inter node were poorly responded. Among the five explants shoot tip has shown more survival percentage.

3.4. Percentage of Aseptic Culture

Significant differences were found among the explants for aseptic culture percentage.

Maximum percentage (80%) of aseptic culture was recorded in case of shoot tips and petiole followed by single node explants (60%). Similar percentage of aseptic culture was seen in case of leaf explants (60%).

S. No	Explants	Contamination %	Aseptic culture %	Survival %
1.	Shoot tip	20%	80%	80%
2.	Single node	40%	60%	60%
3.	Inter node	80%	20%	20%
4.	Petiole	20%	80%	80%
5.	Leaf	40%	60%	60%

TABLE -5: Contamination, aseptic culture and survival percentage of different explants:

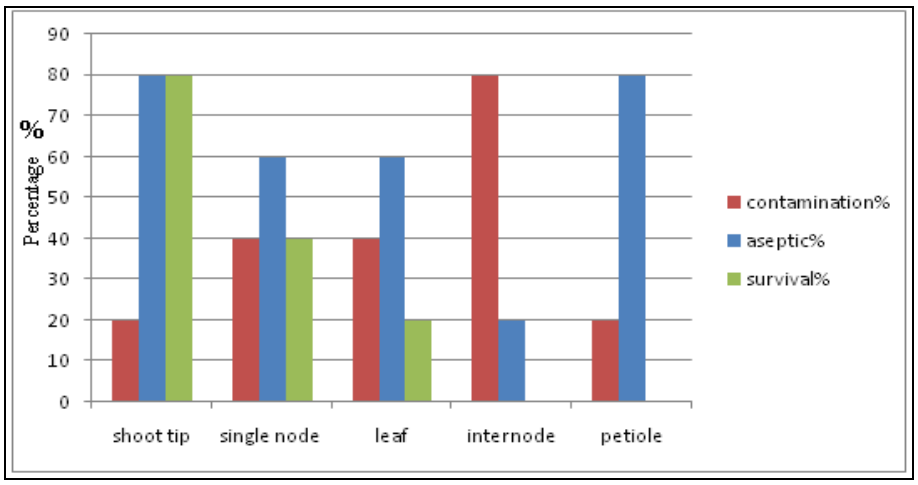

Figure 7: Contamination, aseptic culture and survival percentage of different explants.

Treatments	Explants	
	Shoot tip	Single node
T1- 1mg/l BAP	0	0
T2- 3mg/l BAP	15	0
T3- 5mg/l BAP	11	15
T4-3mg/l 2,4-D	10	9
T5-5mg/l 2,4-D	0	0

Table 6: Number of days taken for shoot initiation, influenced by explant and different growth regulator concentration.

3.5. Influence of Growth Regulator Concentrations on Shoot Regeneration

3.5.1. Number of Days Taken for Initial Sprout (Sprouting)

After the inoculation of explants, it has taken 7 days for sprouting. Shoot tip treated with growth regulator BAP with a concentration of 3mg/l showed initial sprouting after 7 days of inoculation and 10 days for single node treated with growth regulator BAP with a concentration of 5mg/l.

Figure 8: Shoot initiation from shoot tip

3.5.2. Number of Shoots per Explants

The explants shoot tip and single node were responded .There was a shoot proliferation from shoot tip and single node after a week of inoculation .But there was no further shoot multiplication.

Figure 9: Shoot initiation from single node

3.5.3. No. of Days for Callusing

After the inoculation of explants, it has taken 7 days for callusing. Shoot tip treated with growth regulator 2, 4-D with a concentration of 3mg/l showed initial callusing after 7 days of inoculation and 10 days for single node treated with growth regulator 2, 4-D with a concentration of 3mg/l.

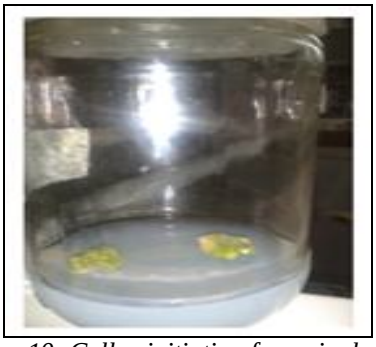

Figure 10: Callus initiation from single node

3.5.4. Shoot Length

The maximum length of the shoot of shoot tip explants was observed and measured as 1.5cm treated with the growth regulator BAP(3mg/l) followed by 1cm treated with the growth regulator BAP (5mg/l). The maximum length of the shoot of the explant single node was observed and measured as 0.5cm treated with the growth regulator (BAP 5mg/l).

Further there was no increase in the length of the shoot tip and no shoot proliferation.

S.No	Treatments(mg/l)	Explants		
		Shoot tip	Single node	Mean
1.	T-1:1mg/l BAP	-	-	0
2.	T-2:3mg/l BAP	1.5cm	-	0.75
3.	T-3:5mg/l BAP	1cm	0.5cm	0.75
	Mean	0.83	0.16	0.5

Table 7: Shoot length (cm) of plantlets as influenced by explant and growth regulator Concentrations

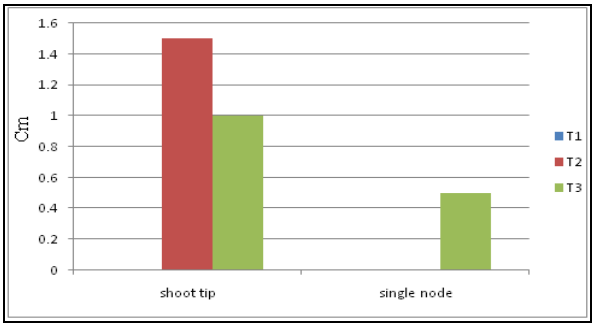

Figure 11: Shoot length (cm) of plantlets as influenced by explant and growth regulator Concentration

S. No	Growth regulator (mg/l)	Response of explants (days)			
		7	10	15	20
1.	BAP-1mg/l	-	-	-	-
2.	BAP-3mg/l	R	S	**S**	-
3.	BAP-5mg/l	R	S	**S**	-
4.	2,4-D-3mg/l	R	-	-	-
5.	2,4-D-5mg/l	-	-	-	-

R= response S=shoot formation - = No response

Table 8: Response of shoot tip explants to different growth regulator concentrations:

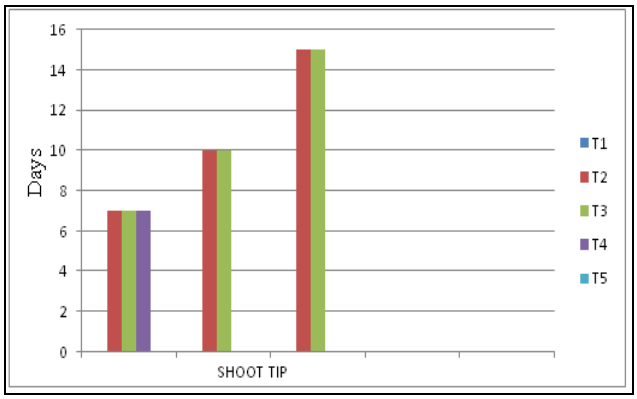

Figure 12: The response of shoot tip explants to different growth regulator concentrations

S. No	Growth regulator (mg/l)	Response of explants (days)			
		10	15	18	20
1.	BAP-1mg/l	-	-	-	-
2.	BAP-3mg/l	-	-	-	-
3.	BAP-5mg/l	**R**	**S**	**S**	-
4.	2,4-D-3mg/l	**R**	-	-	-
5.	2,4-D-5mg/l	-	-	-	-

R= response S=shoot formation - =No response

Table 9: Response of single node explants to different growth regulator concentrations

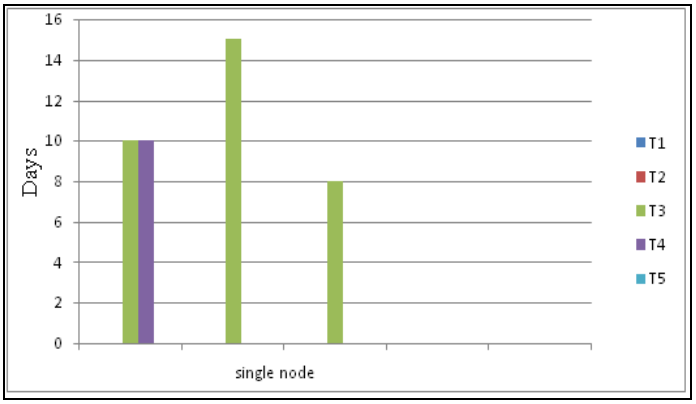

Figure 13: The response of single node explants to different growth regulator concentrations

S. No	Growth regulator (mg/l)	Response of explant(days)			
		10	15	18	20
1.	BAP-1mg/l	-	-	-	-
2.	BAP-3mg/l	-	-	-	-
3.	BAP-5mg/l	-	-	-	-
4.	2,4-D-3mg/l	-	-	-	-
5.	2,4-D-5mg/l	-	-	-	-

Table 10: Response of leaf explants to different growth regulator concentrations

There was no satisfactory result from leaf explants. Shoot tip and single node explants were responded well and there was a shoot formation. But after few days of shoot formation there was a browning of explants and there was no further growth.

4. Discussion

4.1. Hardiness

Even in a greenhouse, the right conditions are tricky to achieve. Sandalwood requires a temperature of 30°C, at least 12 hours of daylight all year round and keeping very dry - the trees only need 3 to 30 cm of rain a year in the wild.

4.2. Propagation

Root suckers from the bases of mature sandalwood trees will transplant successfully, provided there are small 'nurse plants' growing in the area the transplant is intended to grow.

Growing from seed is possible, but very slow. In the wild, birds are probably the chief method of distribution of sandalwood, but the process of breaking dormancy is not yet fully understood.

Some limited success is now being achieved by micro-propagation techniques, but this is still being refined [5].

4.3. Cultivation

Sandalwood depends on other plants for water, trace minerals, still it needs sunlight or 30˚C to synthesize carbohydrates and hence it has green leaves despite having roots attach themselves to the roots of other plants. There are about 300 species of plant that are parasitized by sandalwood.

Cultivated plants grown commercially are often grown in combination with chili pepper (*Capsicum*) and species of Acacia in order to give the trees a host plant. From the base the parasitic roots will extend minimum 10m in each sandalwood tree. The trees required a lot of light to grow easily. [6].

Sandalwood is an endangered species and has medicinal and many other applications. When compared to other methods of growing sandal wood, micro propagation was found to ideal.

4.4. Standardization of Explants

Standardization of explants was done with the explants petiole, leaf, and single node, shoot tip and inter node. The survival percentage of shoot tip, single node and leaf were found to be ideal for micro propagation.

There was a browning of explants soon after surface sterilization. Hence, anti-oxidants like citric acid and Abscisic acid were used. After a few days of inoculation when the explants were observed, most of them were contaminated and some turned into brown colour.

4.5. Leaf as Explants

For the present study the explants of leaf, single node and shoot tip were collected from the garden near REVA University campus, Bengaluru. The trees were five year old. For histological examination a very thin layer of longitudinal section was taken and observed under the microscope. From the longitudinal section the pattern of arrangement of cells was inspected The cells on both the sides of the midrib were found to be small in size and the cells towards middle of the leaf and towards the margin were found to be larger. The pattern is estimated from distal to proximal end. By this investigation, we can say that the cells near the midrib are in a state of active division while the larger cells have matured.

The present histological studies revealed that mitotic activity was localized in the cells near the midrib. Hence, the leaves near the midrib were taken in a longitudinal section and were cut into small pieces and inoculated into the media.

After a few days of incubation, some of them were contaminated and some turned into brown color. The species found in forest are known to be attacked by different pathogen. Phyllosphere is colonized by a variety of epiphytic and endophytic microorganisms [7].

The phyllosphere fungi, primarily relates to the micro environment condition in the leaf surface and their physical, chemical and morphological properties which affect the fungal establishment in the internal tissue [8, 9].

Scientists have carried out studies on endophytic fungi in tree leaves for numerous host plants, when their significance as common symbionts of plants was recognized. Less work is known about the leaves of long lived evergreens [10].

According to Kayini Cladosporium cladosporioides and other fungi like Fusarium oxysporum were the dominant colonizers of forest tree leaves like in Schima walichii. This shows that most of the heartwood trees are infected by endophytic fungi Cladosporium and Penicillium species.

4.6. Single node as Explants

Single node explants collected from the branches of a five year old tree and subjected to surface sterilization and were inoculated into the media. After ten days of incubation there was a shoot formation from the explants treated with the growth regulator BAP with a concentration 5mg/l. But there was no further proliferation from the explants.

4.7. Shoot tip as Explants

The procedure followed for the explants leaf and single node was followed for the shoot tip explants and inoculated into the media.After seven days of inoculation there was sprouting and there was a shoot proliferation.

The growth regulator BAP of 3mg/l and 5mg/l concentrations were responded well. There was no further growth from the explants.

The explants inter node, petiole were not found to be ideal for in vitro micro propagation of explants. Hence, the explants single node, shoot tip and leaf were selected for micro propagation.

Shoot tip and single node were responded well and there was no response from leaf explant. Some of the explants turned brown and some were contaminated.

The growth regulators BAP and 2, 4 – D at a concentration of 3mg/l and 5mg/l were found to be ideal for callusing and direct organogenesis. As there was only a little growth from the explants which were responded, it was concluded that more sterilization and increment in growth regulator concentration may give rise to better growth of the explants.

4.8. Conclusion

The explants shoot tip and single node were found to be standard for direct organogenesis and callusing. Treatment with growth regulator BAP with a concentration of 3mg/l was found to be ideal for shoot tip culture.

Treatment with growth regulator BAP with a concentration of 5mg/l was found to be ideal for single node culture. The explants suitable for callusing were found to be shoot tip and single node with a growth regulator concentration of 3mg/l.

4.9. Scope for Future Research

Better surface sterilization and changes in growth regulator concentration may give better results. Hence this study can be extended with different concentrations of growth regulators to improve the growth of the explants.

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