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"A study on *Santalum album* (an endangered species) for its conservation by the help of Micro propagation strategy "

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ABSTRACT :

Santalum is genus of woody flowering plants, commercially valuable because of its highly valued fragrant heartwood, which contains sandal oil that it used in perfumes, cosmetics, medicines and also in incense sticks industries. Most members of this genus are either trees or shrubs and root parasites which photosynthesize their own food but tap the roots of other species for water and inorganic nutrients. The leaf explants exhibited morphogenetic growth responses and increased many fold in size on MS medium *adjuvant* with various growth regulators *viz*; 2, 4-D, NAA, IAA and IBA, The explants developed into shoots on MS+BAP (5.0 mg/l) and on reduced concentration of BAP (1.5mg/l) multiple shoot production occurred from *in-vitro* raised micro cuttings of *Santalum album* L. Induction of rooting from *in-vitro* raised shoots was achieved on MS medium supplemented with NAA (0.5 mg/l). The most inductive auxin-cytokinin combinations for the production of complete plantlets was BAP (0.5mg/l) + NAA (2.0mg/l) at which explants exhibited best morphogenetic potential in 90% cultures in *Santalum album* L. Micro propagation is a combination of the arts and sciences of plant multiplication in-vitro and plant acclimatization.
Keywords*: Santalum* , Micro propagation, perfumes, cosmetics , inorganic nutrients.

1. INTRODUCTION:

In ancient Egypt, the wood was imported for its medicinal properties as well as for rituals and embalming the dead. Sandalwood played an important role as an economic resource in the kingdoms of Southern India where it was chiefly found. The *Vijayanagar* dynasty expanded during *Krishnadevaraya's* reign due to trade of Sandalwood in early 16th century (*Anonymous, 2003*). *Tippu Sultan,* ruler of the Kingdom of Mysore had declared it a royal tree and monopolised Sandalwood trade in the state in 1792 (*Fox, 2000*). It is part of traditional medical systems such as Chinese

medicine and the Indian healing science known as Ayurveda. Traditionally sandalwood has been used for digestive complications. It is listed as a carminative & digestive muscle relaxant. It has antiseptic properties and has long been valued for treating genito-urinary infections.

"According to *Vamana Purana*, the wood is recommended for worshipping Lord Shiva."

Sandalwood (*Santalum album* L.) is an integral part of Indian culture and heritage. Also well known as the "fragrant gold" of the Indian forest, where it occupies a pre-eminent position, is a very precious and valuable tree (*Rao, et.al 1992*). *Santalum album* L. or Indian sandalwood is a small tropical tree, the most commonly known source of Sandalwood. *Santalum* is genus of woody flowering plants, commercially valuable because of its highly valued fragrant heartwood, which contains sandal oil that it used in perfumes, cosmetics, medicines and also in incense sticks industries (*Srinivasan, et.al 1992*). *Santalum album* L. grows as a tall or small tree, 4-6 m (12–20 ft) high and 2–4 m (7–12 ft) wide. The rough bark is dark grey and the branches ascending in character. Smaller plants formed by suckers from the roots are sometimes found surrounding larger plants. Its use in treatment of skin problems is legendary (*Ghani N. Khazainul Advia, 2007*). The warm, sweet slightly spicy precious wood notes present a melodic blend which is at once distinct yet not over powering (*Kumar, et al, 2012*).

2. METHODOLOGY :

In the present study an attempt was made for standardized protocol for direct shoot proliferation from meristemetic and nodal explants taken from apical as well as axillary buds of twigs of *Santalum album* L. The excised explants were sterilized by rinsing in running tap water to remove dirt and pathogens for 45 min, then washed 4-5 times with Double Distilled Water (DDW), and then treated the explants with Extrain (2%). Next were treated explants with (2%) Bevistin solution for 10 minutes. The inoculation cabinet (Laminar Air Flow) was first wiped clean with 70% ethanol and sterilized using germicidal ultraviolet for 10-15 min. All metal instruments, glassware's and other accessories were steam sterilized for 20 min at 121°C with 15 lb/inch^2 normal stream pressure in an autoclave. The MS medium described by *Murashinge and Skoog's* (1962) were used as basal medium throughout this investigation. Preparation of basal MS medium followed by addition of different growth regulators

in different concentrations to prepare different media formulates, followed by addition of agar substitute in concentration of 8mg/l following and stream sterilization of culture tubes or flasks having media in an autoclave.

2.1. CALLUS CULTURE AND PLANT REGENERATION:

In this route explants were treated with different concentration of 2-4D, for induction of callus in aseptic condition. Callus were treated with different Growth regulator for plant regeneration. The cultures were maintained in culture tubes and conical flasks and were kept in the culture room at a temperature of 25± 2°C, relative humidity (RH) of 60-70% and a light intensity of approx. 2500 lux. Provided by cool, white, fluorescent tubes under a photoperiod of 16/8 hr (light/dark).

2.2. PREPARATION OF STOCK SOLUTIONS

STOCK-I (20X) Macronutrients:

COMPOUND	AMOUNT (mg/l)
NH_4NO_3	33000
KNO_3	38000
$CaCl_2.2H_2O$	8800
$MgSO_4$	7400
KH_2PO_4	3400

STOCK-II (200X) Micronutrients:

COMPOUND	AMOUNT (mg/l)
KI	166
H_3BO_3	1240
$MnSO_4.4H_2O$	4460
$ZnSO_4.7H_2O$	1720
$NaMoO_4.2H_2O$	50
$CuSO_4.5H_2O$	5
$CaCl_2.6H_2O$	5

STOCK-III (200X) IRON:

Compound	AMOUNT (mg/l)
$FeSO_4.7H_2O$	5560
$Na_2EDTA.2H_2O$	7460

STOCK-IV (200X) VITAMINS:

Compound	AMOUNT (mg/l)
INOSITIOL	20,000
NICOTINIC ACID	100
PYRIDINE HCl	100
THIAMINE	20
GLYCINE	400

2.3. METHOD OF FRESH CULTURING:

(OPEN LAB):

Collection of explants from the field

⇓

Kept it in a bottle covered with muslin cloth

⇓

Keep it under running tap water to remove dirt and pathogens for 30 minutes-45 minutes.

⇓

Wash it 4-5 times with Double Distill Water (DDW)

⇓

Treat the explants with Extrain (2%) for 15 minutes to kill the bacteria and other pathogens

⇓

Wash it 5-6 times with DDW

⇓

Now treat the explants with (2%) Bevistin solution for 10-15 minutes

⇓

Wash it 4-5 times with DDW for the complete removal of Bevistin

⇓

Transfer the bottle to Laminar Air Flow chamber

(CLOSE LAB):

Wipe LAF and glass wares with alcohol

⇓

LAF cabinet and glass wares are sterilized under UV light for at least 45minutes-1hour

⇓

Keep the washed explants and all needed apparatus like 8 sterile DDW bottles, required media, forceps, and scalpel; under UV light in LAF for 40 minutes

⇓

Now transfer it in pre-sterilized bottle with the help of pre-sterilized forceps

⇓

Wash it with 4-5 times with sterilized DDW

⇓

Add 0.1% $HgCl_2$ solution and keep it for 2-5 minutes (time may vary with the explants)

⇓

Wash it 4-5 times with sterile DDW again

⇓

Explants are ready for inoculation

3. OBSERVATION:

3.1. Chemical Sterilization of Explants

TABLE No. 1; Effect of $HgCl_2$ (0.1%) treatment period on sterilization of nodal segments of *Santalum album* L. excised from field grown mature trees.

Treatment Duration (min)	No. of explants	Rate of contamination (after days of treatment)					% of contamination free explants after 10 days.
		2nd	4th	6th	8th	10th	
3	10	-	3	5	9	10	10
4	10	-	-	2	3	5	50
5	10	-	-	-	1	2	70

TABLE No. 2 :Effect of $HgCl_2$ (0.1%) treatment period on surface sterilization of leaves of *Santalum album* L. excised from field grown mature trees.

Treatment Duration (min)	No. of explants	Rate of contamination (After days of treatment)						Percentage of contamination free explants after 10 days.
		2nd 4th		6th 8th		10th 12th	16th	
2	10	4	5	6	7	10	10	0
3	10	-	-	-	5	10	10	0
4	10	-	-	-	-	-	-	100
5	10	-	-	-	-	-	-	100*

TABLE No. 3 : Effect of BAP (mg/l) on shoot multiplication from *in vitro* raised micro cuttings of *Santalum album* L.

BAP (mg/l)	% age of culture forming usable shoots	Average no. of shoots / explant	Average length of shoots (cm)
5.5	30	2.0	2.2
4.5	30	3.0	2.3
3.5	40	4.1	2.5
2.5	70	7.1	2.4
0.5	80	8.0	2.3
1.5	90	10.2	2.8

TABLE No. 4 : Effect of auxin (mg/l) on root induction from *in-vitro* raised shoots of *Santalum album* L.

Auxins (mg/l)	% of cultures forming useful roots	Average no. of roots/ explant	No. of days taken for rhizogenesis
IAA			
0.5	40	3.1	12
1.0	50	3.5	10
IBA			
2.0	20	3.0	18
3.0	20	3.1	18
NAA			
0.5	60	5.3	16
1.7	60	5.2	16
1.0	70	3.6	10
0.5	80	7.5	6

3.2. In-vitro response of shoot tip explants

TABLE No.5: Effect of cytokinin (mg/l) on shoot multiplication from shoot tip explants of *Santalum album* L.

Cytokinin (mg/l)	%age of cultures forming usable shoots	Average no. of shoots/explants	No. of days taken
MS basal medium control			
Kn			
0.5	20	4.0	20
1.5	30	5.3	25
BAP			
0.2	50	10.1	12
1.0	60	25.4	10
2.0	80	40.2	6

TABLE No.6: Effect of auxins on root induction in *in vitro* grown micro cuttings of *Santalum album* L.

Auxins (mg/l)	%age of cultures forming usable roots	Average no. of shoots per/ explant	Average length of roots (cm)
IAA			
0.5	30	2.0	1.0
1.0	40	3.2	2.0
IBA			
0.5	50	3.5	2.0
2.0	60	4.3	2.1
NAA			
0.2	60	5.2	2.2
0.5	70	6.3	4.4
1.0	80	8.2	5.1

TABLE No. 7 : Effect of PGR's (auxin-cytokinin combination) on shoot tips explants of *Santalum album* L.

PGR's (mg/l)	%age of cultures regenerated	Average no. of shoots/ explant	Average no. of roots/explants
BAP+IAA			
2.0+0.2	20	2.3	3.2
5.0+0.5	30	1.5	1.0
BAP+IBA			
0.2+0.1	20	2.0	1.0
1.0+0.5	30	2.7	2.1
BAP+NAA			

2.0+0.2	70	8.3	6.3
5.0+0.5	80	10.2	8.1
Kn+IAA			
0.5+0.2	20	3.1	3.0
1.0+0.2	20	2.0	1.1
Kn+IBA			
0.2+0.5	20	3.2	2.0
1.0+2.0	20	2.1	1.1
Kn+NAA			
2.0+0.5	20	3.0	2.1
0.5+0.2	20	2.2	2.0

4. CONCLUSION AND DISCUSSION:

Based on the results achieved in the present study the main conclusions are as cultures of *Santalum album* L. required an initial dark period of about 72 hours to establish. Thus the florescent light has an definite impact on establishment of cultures , Growth regulators *viz*; auxins and cytokinins were found to be responsible for inducing dedifferentiation in the mature differentiated plant tissues. The leaf explants exhibited morphogenetic growth responses and increased many fold in size on MS medium *adjuvant* with various growth regulators *viz*; 2, 4-D, NAA, IAA and IBA, The explants developed into shoots on MS+BAP (5.0 mg/l) and on reduced concentration of BAP (1.5mg/l) multiple shoot production occurred from *in-vitro* raised micro cuttings of *Santalum album* L. Induction of rooting from *in-vitro* raised shoots was achieved on MS medium supplemented with NAA (0.5 mg/l). The most inductive auxin-cytokinin combinations for the production of complete plantlets was BAP (0.5mg/l) + NAA (2.0mg/l) at which explants exhibited best morphogenetic potential in 90% cultures in *Santalum album* L. Finally it was concluded that MS Basal medium + 2,4-D is a suitable medium for callus forming in case of explants. BAP proved to be best for shoot differentiation and NAA is the best auxin for rhizogenesis. Among from the inducers IAA help bitterly in induction of rooting but in case of *Santalum album* L. NAA proved very effective auxin for root initiation.

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