Advantages of Micro propagation of Tree Biotechnology: An economic and medicinal evaluation

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Abstract: *Dalbergia sissoo* (Shisham) belongs to sub-family Papilionoidae of the Fabaceae family. It is an important tree for timber, fooder and fuel wood. It is one of the major tree species under social forestry programme, but its mortality is being observed that compel farmers to give up the Shisham plantation. The forest trees are natural gift and divine habitat that makes the country healthy and wealthy. Shisham is an important renewable natural resources. It provides several forest products like fuel, timber, lumber. Paper, fooder etc.

Micropropagation has a great potential to improve traditional methods of tree breading. It reduces the time to produce new varieties. Micropropagation is very useful for mass clonal propagation of *Dalbergia sissoo* or any forest trees. *In vitro* the rate of multiplication cannot be expected by any of the *in vivo* methods of clonal propagation. Multiplication cycle is very short. In micropropagation, plant multiplication can continue throughout the year irrespective of the season. It is also feasible to preserve just the germlasm in a deep freeze (cryopreservation) as mass of the cells & later grow a complete plant in tissue culture. So, millions of potential forests trees besides shisham could be stored in a few test tube. The culture leaves showed swelling and good callus formation particularly on media with 2, 4-D and BAP. Stem culture resulted in more callus formation and more organogenesis than leaf culture. Caulogenesis was more common than rhizogenesis and normally occurred through the formed calli. The best media caulogenesis was with 2, 4-D (1.1 mg/l) and BAP (5.5 mg/1). Thus, the *in vitro* plants were grown in the field. An analysis of characteristics of tissue cultured plants has shown that some characters such as leaf size and colour showed some changings. Thus, the experiments and the morphogenetic observation had shown that the important timber, fodder and fuel wood plant of Fabaceae family *Dalbergia sissoo* could be micropropagated and improved upon through micropropagation. The ministry of environment and forest constituted the National afforestation and Eco development board (NAEB) in August 1992 for afforestation and management strategies. It has been taken into consideration.

Index Terms: Tree Biotechnology, Social Forestry Programme, Morphogenetic studies, Caulogenesis, Callus, *Dalbergia sissoo*.

INTRODUCTION

Dalbergia sissoo (Shisham) belongs of the Fabaceae family. It is an important tree for timber, fooder and fuel wood. It is one of the major tree species under social forestry programme, but its mortality is being observed that compel farmers to give up the Shisham plantation.

The forest trees are natural gift and divine habitat that makes the country healthy and wealthy. Shisham is an important renewable natural resources. It provides several forest products like fuel, timber, lumber. Paper, fooder etc. Ecologically, it regulates the level of rainforest like other forest plants which is necessary for existence of vegetation on the earth. It also checks flood, drought & soil erosion.

Micropropagation has a great potential to improve traditional methods of tree breeding. It reduces the time to produce new varieties. Micropropagation is very useful for mass clonal propagation of *Dalbergia sissoo* or any forest trees. *In vitro* the rate of multiplication cannot be expected by any of the *in vivo* methods of clonal propagation. Multiplication cycle is very short. In micropropagation, plant multiplication can continue throughout the year irrespective of the season. It is also feasible to preserve just the germlasm in a deep freeze (cryopreservation) as mass of the cells & later grow a complete plant in tissue culture. So, millions of potential forest trees besides Shisham could be stored in a few test tube. The ministry of environment and forest constituted the National afforestation and eco development board (NAEB) in August 1992 for afforestation and management strategies. The forest management and microplanning to develop specific afforestation and eco-development package for augmenting biomass production is the micropropagation of plants. In the forest sector, the dwindling resources of forest wealth and the increased demand for wood and wood products calls for urgent effort to regenerate forest and in this regard, tissue culture has great potential. So tissue culture studies of *Dalbergia Sissoo* were properly used for morphogenetic studies at all levels of differentiation, regenerations and field transfer.

Thus, to explore the possibility of micropropagating *Dalbergia sissoo* in which, there are no previous reports of success of morphogenetic studies in tissue culture of various vegetative organs were done. The studies were done at different levels of callus formation, differentiation, regeneration of plantlets and their field transfer and establishment. More than 2 lakhs plants of *Dalbergia sissoo* can be also easily obtained from a single bud in a year- an established protocol can be used for social forestry.

MATERIALS AND METHODS

The seed, nodal and internodal segments collected from different superior clones were used as explants for axillary bud proliferation & callus formation. The different clones were selected for micropropagation from the different places of town, Muzaffarpur, Bihar. The curiosity to investigate the *in vitro* proliferation pattern, multiplication rate, rooting response of different clones & regeneration of plantlets for social forestry. The present study dealt with the standard protocol for micropropagation of *D. sissoo* from the selected superior clones to distinguish the physico-chemical interaction, callus formation & regeneration of plantlets through the different culture media & hormonal regulations

Methods

STERILIZATION OF EXPLANTS: ***Seeds,*** **nodal & internodal segments (measuring 4 -7 cm containing axillary buds) were first surface sterilized using 0.1% HgCl2 for 15 min, after which they were washed with sterile distilled water 3-4 times for further inoculation in medium.**

EFFECT OF PHYTOHORMONES & THEIR INTERACTION: To study the effect of Cytokinins on axillary bud proliferation & mass callus propagation from the nodal and internodal segments (collected from the selected three superior clones), BAP& KN (Kinetin) were added in full strength MS medium at concentrations (mg/l)) of 0.1 to 5.5 and 0.1 to 5.5 separately. The superior clones were inoculated on full strength MS medium supplemented with combinations of cytokinin, BAP (0.1-5.5 mg/l) and auxin 2,.4-D at varying concentrations (0.1 to 5.0 mg/l) to observe the effect of cytokinin-auxin combination on axillary bud proliferation & mass callus proliferation from nodal & internodal regions.

HARDENING AND ACCLIMATIZATION OF PLANTLETS*: Generally, regenerated plants need proper hardening and acclimatization. Rooted shoots from four week old cultures were transferred to soil under shade house after hardening. For hardening, the plantlets were taken out from the flasks, washed to remove adhered agar and then transferred to autoclaved 250 ml screw cap glass bottle containing 1/3 volume of vermiculite. These plantlets were nurtured with half strength MS medium (without organics) twice a week for two weeks and were then kept in tissue culture room. After two weeks, these bottles were shifted to a mist chamber having relative humidity of 80-90% with a temperature of 25 ± 5°C.. After one month in shade area of lab, the plants were transferred to bigger polybags/pots containing same soil composition and were irrigated with tap water. Plants were further kept in shade house for two months. During *in vitro* studies is general, cultured cells are not genetically stable. Polyploidy, aneuploidy and chromosomal changes in cells cultured under various conditions have been studied and described. The frequency of plantlets that survived during acclimatization was about 13 to 18%.

Thus, the present work has established to regenerate plants of *D. sissoo* in only few weeks to promote social forestry at very low cost as compared to the traditional supply of Shisham plants.

RESULTS & DISCUSSION

The seed, & stem explants were used for callus formation as well as the regeneration of plant through the micropropagation. The seeds are surface sterilised before scarification. Similarly, the stem explants were taken from the developing young shoots and size ranging between 4 to 7 cm roughly. The overall tissue culture response for callus formation, for callus growth, for regeneration; the cultures stem explants gave the best response. Most of the stem explants showed slight swelling in the first week of culture. The whole explant or callus gave good callusing or embryonic stages. The nodal part of stem gave mass proliferation of callus with 2,4-D (1.1mg/l) and BAP (1.1 mg/l) in 12-18 days. The internodal (soft) parts of stem gave more mass proliferation of callus with 2,4-D (1.1 mg/l) and BAP (5.5 mg/l) in 14-25 days. Thus, the stem explants gave the best result as compared to seed/cotyedonary culture.

The nodal and internodal callus were cultured on the concentration of 2,4-D(1.1mg/l) and BAP (5.5 mg/l). The callus became yellowish to reddish in colour. The callus became embryogenic. If the concentration of cytokinins (KN and BA) was increased in sub - culturing medium, more callus formation and regeneration of plant were obtained. BAP gave better result for callusing than KN.

RESPONSES OF EXPLANTS AT DIFFERENT CULTURE MEDIUM		
TABLE 1.1: PLANT HORMONES SELECTED FOR MICROPROPAGATION OF *DALBERGIA SISSOO*		
Adjuvants (Phytohormones)		Used Concertation (mg/l)
1. Auxin	2,4-D	0.1 0.5 1.0 1.1 5.0
2. Cytokinins	KN (kinetin)	0.1 0.5 1.0 1.1 5.0 5.5
	BAP (Benzylaminopurine)	0.1 0.5 1.0 1.1 5.0 5.

TABLE 1.2: CALLUS GROWTH		
1.	-	No growth
2.	+	Low growth
3.	++	Moderate growth
4.	+++	Good growth

TABLE 1.3: SELECTION OF MEDIA			
Sl. No.	Media	Selection of Media	Main response
1.	M1	MS + 2, 4-D (1.00 mg/l) + KN (0.1mg/l)	Organogenesis and Caulogenesis.
2.	M2	MS + 2,4-D (1.1 mg/l) + BAP (1.1mg/l)	Organogenesis and Caulogenesis.
3.	M3	MS + 2,4-D (1.1mg/l) + BAP (5.5 mg/l)	Organogenesis and Caulogenesis.
4.	M4	MS + 2,4-D (1.1 mg/l)	-
5.	M5	Coconut water	Organogenesis

TABLE 1.4 SEED CULTURE OF *DALBERGIA SISSOO*			
RESPONSE			
SHOOT FORMATION			
Medium	(i) % Culture showing shoot formation	(ii) No. of shoot/ culture	(iii) Shoot growth
M1	-	-	-
M2	50	40	+
M3	60	57	++
M4	-	-	-
M5	80	60	+++

TABLE 1.5 SEED CULTURE OF *DALBERGIA SISSOO*			
RESPONSE			
SHOOT FORMATION			
Medium	(i) % Culture showing shoot formation	(ii) No. of shoot/ Culture	(iii) Shoot growth
M1	-	-	-
M2	25	18	+
M3	30	20	++
M4	-	-	-
M5	20	15	+

TABLE 1.6 SEED CULTURE OF *DALBERGIA SISSOO*		
RESPONSE		
EXISTING SHOOT DEVELOPMENT		
Medium	(i) % Culture showing shoot Development	(ii) Shoot growth
M1	50	+
M2	60	+ +
M3	70	+ + +
M4	–	–
M5	60	+ +

TABLE 1.7: NODAL STEM CULTURE OF *D. SISSOO*					
Response Medium	M1	M2	M3	M4	M5
1. Callus Formation					
(i) % Culture showing callus formation	-	80.00	85.00	-	-
(ii) Callus growth	-	+ + +	+ + +	-	-
(iii) Callus colour	-	White	White	-	-
(iv) Callus nature	-	Compact	Compact	-	-

TABLE 1.8: INTERNODAL STEM CULTURE OF *D. SISSOO*					
Response Medium	M1	M2	M3	M4	M5
1. Callus formation					
(i) % Culture showing callus formation	-	82.00	85.00	-	-
(ii) Callus growth	-	+ + +	+ + +	-	-
(iii) Callus colour	-	White	White	-	-
(iv) Callus nature	-	Compact	Compact	-	-

OBSERVATION OF MICROPROPAGATION OF DIFFERENT EXPLANTS OF SEED

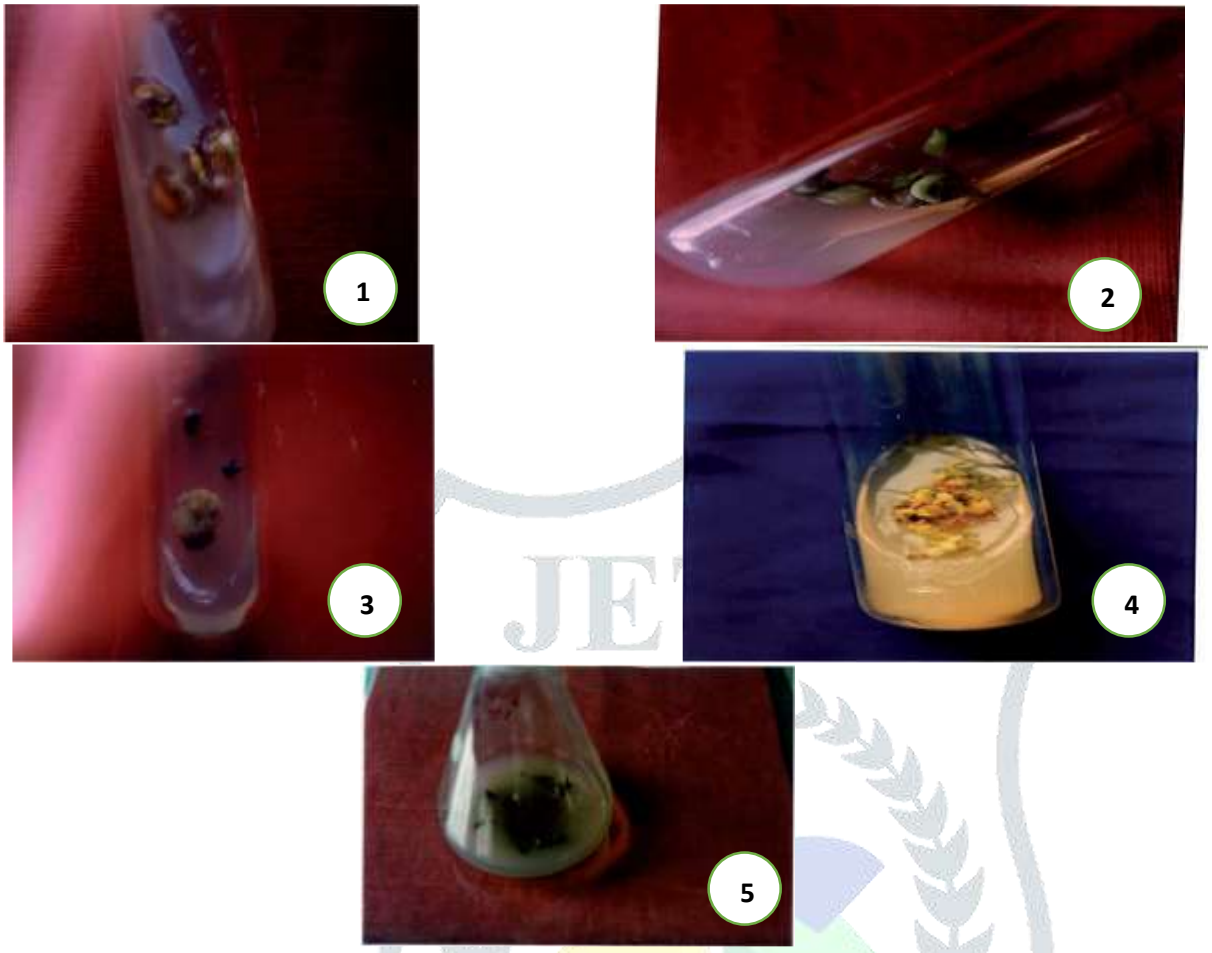


Fig -1: Germination of seed of ***Dalbergia sissoo*** on MS-medium + 1.1mg1-1 2,4- D+0.1 mg1-1 KN (18 days old culture)

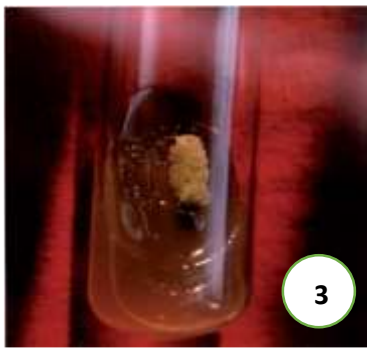
Fig -2: Germination of seed of ***Dalbergia sissoo*** on MS-medium + 1.1mg1-1 2,4- D+5.5 mg1-1 BAP (5 days old culture)

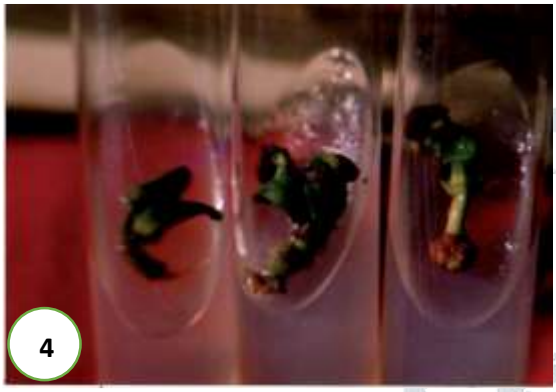
Fig -3: Seed culture of ***Dalbergia sissoo*** showing mass proliferation of callus on MS-medium + 1.1mg1-1 2,4-D+5.5 mg1-1 BAP.

Fig -4: Shoot apex culture of ***Dalbergia sissoo*** showing callus on MS-medium + 0.1mg1-1 2,4-D+5.5 mg1-1 BAP (15 days old culture)

Fig -5: Germination of seed of ***Dalbergia sissoo*** on coconut water (18 days old culture)

OBSERVATION OF MICROPROPAGATION OF DIFFERENT EXPLANTS OF STEMS





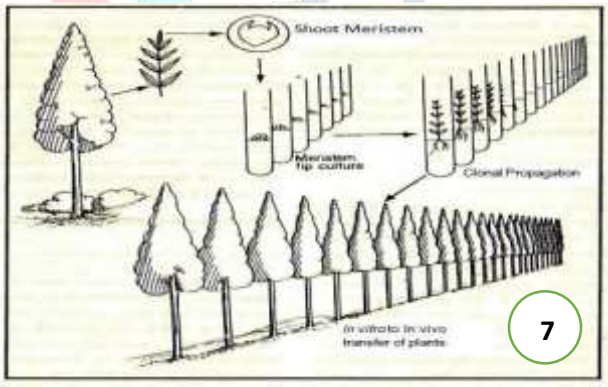


Fig -1: Nodal stem culture *of* ***Dalbergia sissoo*** on MS-medium + 1.1 mgl-1 2,4-D+ 1. 1mgl-1 BAP, (12 days old culture)
Fig -2: Nodal stem culture *of* ***Dalbergia sissoo*** on MS-medium + 1.1 mgl-1 2,4-D+ 1.1mgl-1 BAP (18 days old culture)
Fig -3: Internodal culture of ***Dalbergia sissoo*** on MS-medium + 1.1 mgl-1 2,4-D+ 5.5mgl-1 BAP showing mass proliferation of callus culture (20 days old culture).
Fig -4: ***In vitro*** regenerated plant culture of ***Dalbergia sissoo*** after 20 days on MS-medium + 1.1 mgl-1 2,4-D+ 5.5mgl-1 BAP showing callus formation from root, stem and leaf.
Fig -5: Regenerated plant of ***Dalbergia sissoo*** on MS medium + 0.1 mgl-1 KN+1.1 mg-1 2,4-D in soil.
Fig -6: Regenerated Plant of *Dalbergia sissoo* grown in the field.
Fig -7: Mass production of Regenerated Plants as social forestry with high economic value

ADVANTAGES OF *DALBERGIA SISSOO* THROUGH RAPID MULTIPLICATION

When a new method such as micropropagation is replacing an existing and successful propagation procedure within the industry, there must be distinct and clear advantages. These are rapid multiplication rate of easily regenerated species. Shoot tip culture can provide 5-10 axillary shoots every 4 weeks for each original bud. The cycle of axillary bud excision and shoot multiplication soon provides very large numbers of plants. Production of difficult-to-propagate plants so that new varieties and species can be introduced to the market.

MEDICINAL ADVANTAGES OF *DALBERGIA SISSOO*

Various parts of *Dalbergia sissoo* are used for treating several diseases, in Ayurveda. The leaves are used for eye pain, swelling, painful urination, gynaecological disorder etc. Leaves & bark are used as astringent in bleeding disorders. The paste of leaves mixed with sweet oil is used in skin excoriation i.e. abrading of skin. It is noticed by many scientific studies that the tree of *Dalbergia sissoo* is anti-inflammatory, antinociceptive or reducing sensitivity to painful stimuli property, antidiabetic, analgesic & antioxidant.

AN ECONOMIC EVALUATION

Micropropagation of forest plants have been developed by which about 500 plants of teak & 1,00,000 plants of *Eucalyptus citriodora* can be obtained from a single bud in a year. Similarly, more than 2 lakhs plants of *Dalbergia sissoo* can be also easily obtained from a single bud in a year.

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