Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Investigation of micro-propagation via callus induction from different explants in case of a recalcitrant plant species: Chandan (Sanctum album L.)

Chandni Upadhyaya

P. M. Patel Institute of Bioscience, Sardar Patel University, Anand 388001, Gujarat
Email: chandni.upadhyaya196@gmail.com

ABSTRACT:

In a view of commercial products requirement across the globe is it extremely important to preserve the Sanctum album species. Sanctum album, also known as Chandan in India, has been commercially exploited significantly and hence the extinction of species is threatened. To sustain the ecosystem it is necessary to preserve these plant species. Biotechnology through micro-propagation via callus induction provides effective and viable means of conserving sanctum album species. This paper provides the investigations of micro-propagation through the help of callus induction technique for *Sanctum album L.*

***Corresponding author**

Chandni Upadhyaya

P.M.Patel Institute of Bioscience,

Sardar Patel University, Anand 388001, Gujarat

Email: chandni.upadhyaya196@gmail.com

INTRODUCTION:

Sandalwood belongs toSantalaceae family. This family group is comprising of 29subgroups-taxonomic category which is having more than 350 various species out which 15+ species belong so Santalum genus. Out of 350+ species around 15 species are in existence which are available in Asia Pacific region.There is multiple diversity of this species. As per the Plant list given in year 2015 there are 12 species names are accepted and remaining 41 species names are not resolved. These discrepancies suggest that some attentionto the taxonomy of the Santalum genus is required[1].

In the economic manner, most vital species are Indian, East Indian and Australian Sandalwood out of multiple species of Sandalwood. Indian traditional medicine has alot of importance of Sanctum Album L. in addition to its utilization in perfumes, aroma liquid manufacturing, worshiping sculptures, home and office futures[2-3]. The sandalwood oil is also being utilized for herbal treatment and cosmetic products for long time now in India and Egypt. In a historical view point, by understanding the importance of sandalwood, Nizam of Mysore Tippu Sultan announced sandalwood as a royal tree. The sandalwood trade started from India in late 18th century. Initially, sandwood incense were quite popular and hence the trade increased significantly by mid-19th Century. This economical utilization of sandalwood created scarcity of the sandalwood in the region and eventually sandalwood started getting extinct. The heartwood in Sanctum Album typically comes in existence after certain maturity[3-5]. The trees in early period of life does not have heartwood. The expected rich heartwood appear after 25 years of existence of Sanctum album tree and then after it can provide the necessary oil to be utilized for aromatic utilization[6-8]. The concentration of the aromatic oil is different as the tree varies, however, concentration is typically lesser than 5%in majority of the Sanctum album L. trees[9-10]. The USFDA and CoE have identified essential oil of Sanctum Album L as safe use in food product[11].

Due to immense utilization of Sanctum album based commercial products, the sandalwood stock available in nature has gone done significantly which in turn has raised the cost of the wood against the market demand[12-13]. Additionally, illegal cultivation of the sanctum album wood it is difficult to estimate the exact supply and demand ratio. As per the estimates thousands of tonnes of trade of Sanctum album wood is occurring per year and it is extremely important to put more efforts for production of sandalwood.

Natural production of the Sanctum Album L. happens with the help of plant seeds and root suckers. The in-vitro propagation focuses on reproduction from various explants such as hypocotyls, zygotic embryo, and somatic embryogenesis by means of a callus phase[13].

MATERIALS AND METHODS:

Out of majority of the in-vitro examination carried out on Sanctum album L. tissue culture and micro-propagation are quite famous techniques for large-scale propagation.In-vitro tissue culture provides sterile culture environment and also permits the examination of stage-wise development. There are five stages of in-vitro propagation namely: Culture Initiation, multiplication, rooting, acclimatization and field establishment.

For the proposed experiments, explants were taken from a thirty-year old stock maintained in the farm. Fungicide (Tilt, 5 mL/L) was twice every week to minimize the contamination in cultures. Topsin (4 g/L) and Thiram (1.5 g/L) were sprinkled alternatively. Albert solution (45.0 mL per plant) was given on every week. The explants were collected at dawn and washed in soap water for 10-15 minutes and then cleaned for 45 minutes under clean running water.These were further immersed in CaptanTm (1.5 g/L) for around half an hour.Then after they were dipped in 10% CloroxTm for around 20 minutes, then after in ethanol for 15 minutes and then in distilled water cleansing was carried out.

MS medium was utilized as the basal medium. The pH value was kept at 5.5.To reduce the expenses per plant, commercial jelly moss (7.5 g/L) was utilized as a solidifying operator. Cultures were incubated at26 ± 2 ºC. CRD was utilized for testing in around 10 replicates.

Optimum explant Source for Callus Induction

For the selection of best explant source for callus initiation, 2.0 cm long nodal segments (2nd and 3rd nodal segment from the meristem) and mature and immature seeds (where the pericarp is green and still attached to the plant and leaf discs) (1.0 cm^2) were used. After surface sterilisation they were cultured in MS medium supplemented with 2.5 mg/L 2,4-dichloro phenoxy acetic acid (2,4-D) and 3.0 mg/L kin and incubated in the dark at 25 ± 1 °C. Growth regulator free MS medium was used as the control. Mean callus diameter and the percentage of callus production were measured. Data were collected at 2 week intervals.

RESULT AND DISCUSSION:

Effect of growth regulators on callus induction

The MS basal medium supplemented with auxin (NAA) and cytokinins (Kn or BA) in combinations with 30g/l sucrose were used for initiation and proliferation of callus from stem, mature, semi mature and immature seeds, inflorescence and flower buds explants of Santalum album L.. The medium devoid of growth regulators did not favour callus initiation even when the cultures

were kept for prolonged period. Induction of callus was observed in mature and semi mature seeds of S. album after 4-5 week of culture. However, the immature inflorescence and flower buds did not show any kind of growth. Though the immature seeds, showed response for callusing after 3-week of culture, however, the initiated calli became dry. Auxin like NAA in combination with BA or Kn favored callus induction from stem, mature and semi mature seeds explants. The cytokinins, BA or Kn (0.00, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 mg/l) in combination with NAA (0.0, 0.01, 0.10 mg/l) induced white callus within 4 weeks of culture in stem explants as shown in figure 1; white and brownish callus was found in seed explants as shown in Figure 2. However, callus growth was maximum in the half strength MS medium containing BA and NAA in combination with GA3 which promoted compact callus formation in stem and seed explants within 20-28 days of culture. Best callusing was observed on half strength MS medium containing 3.0 mg/l BA and 0.1mg/l NAA along with GA3 0.5mg/l and Ads 20mg/l as additives which is the best media tested for callusing as shown in Table 1.

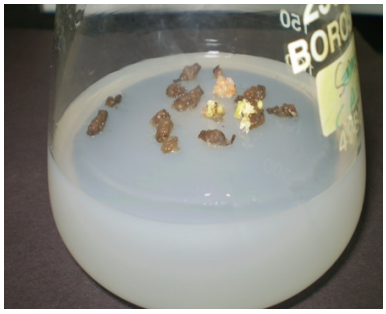

Fig. 1. Induction of callus from stem expalnt of *S. album*

Fig.2. Induction of callus from semi mature and mature seed explant of S. album

Table 1. Effect of growth regulators on callus induction of *Santalum album L.*

% of Culture response		
Source of explant		
Stem (Mean ± S.E)*	**Semi mature seeds (Mean ± S.E)***	**Mature seeds (Mean ± S.E)***
0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3.0 ± 0.06	10.3 ± 0.09	6.3 ± 0.13
13.8 ± 0.02	33.8 ± 0.02	20.2 ± 0.05
0.0 ± 0.0	8.0 ± 0.13	6.8 ± 0.03
6.6 ± 0.04	60.6 ± 0.20	9.4 ± 0.02
6.8 ± 0.03	62.8 ± 0.06	12.3 ± 0.10
15.3 ± 0.13	65.3 ± 0.03	15.2 ± 0.04
20.4 ± 0.06	70.4 ± 0.03	10.8 ± 0.16
70.3 ± 0.13	80.3 ± 0.09	25.3 ± 0.10

*Mean of 10 explants per treatment; experiment repeated twice

For good quality callus, after getting callus in primary culture, it was transferred after each 4 week interval in to the fresh callusing medium.

Shoot Induction From Callus

Calli derived from stem explants were shifted to the MS medium augmented with various concentrations of cytokinins like BA as well as Kn (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) with or without the compounding of auxin like NAA, showed shoot buds induction as shown in Figure 3and calli raised from seed explant produced shoot buds as shown in Figure 4 and Figure 5. Among all the media tested, the MS medium augmented with Kn, gibberellic acid and adenine sulfate was analyzed to be optimum media for shoot reproduction. Effect of growth regulator is shown in table 2.

Fig. 3. Shoot induction from calli of stem explants

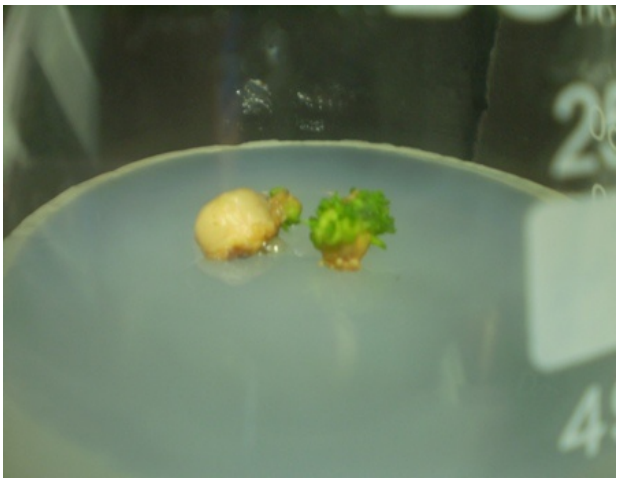

Fig. 4. a Shoot buds initiated fromcalli derived from semi-matureseeds

Fig. 4 b. Shoot induction from calli derived from mature seed

Fig. 5. Calli derived fromsemi mature seed showed shoot multiplication from calli after 8 weeks of culture

Table 2.Analysis of growth regulator effect on shoot induction from callus of *S. album*

% of Culture response		
Source of explant		
Stem (Mean ± S.E)*	**Semi mature seeds (Mean ± S.E)***	**Mature seeds (Mean ± S.E)***
0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3.0 ± 0.02	10.3 ± 0.03	6.3 ± 0.03
20.8 ± 0.10	23.8 ± 0.02	18.2 ± 0.05
10.0 ± 0.06	8.0 ± 0.05	6.8 ± 0.03
68.6 ± 0.10	71.6 ± 0.09	9.4 ± 0.02
30.8 ± 0.03	25.8 ± 0.03	12.3 ± 0.06
15.3 ± 0.03	5.3 ± 0.05	5.0 ± 0.04
20.4 ± 0.06	10.4 ± 0.06	11.8 ± 0.09
60.3 ± 0.03	70.3 ± 0.03	40.3 ± 0.13

*Mean of 10 explants per treatment; experiment repeated twice

Mature Seed Germination With Multiple Shoot Induction

Variouskinds and concentrations of cytokinins and auxin such as BA (2.0, 3.0 mg/l), NAA (0.01 and 0.1 mg/l) and Ads (20 and 50 mg/l) were incorporated in ½ MS media for mature seed germination of *S. album*. Mature seeds germinated in ½ strength MS supplemented with 3.0 mg/l BA, 0.1 mg/l NAA and 0.5 mg/l GA_3 after 5 week of culture as shown in Figure 6. After germination, multiple shoots (10-12 numbers of shoots) were arisen from the germinated seeds within 6-7 week of germination in the media used for germination as illustrated in Figure 7.

Fig. 6. Germination of mature seed of *S. album*.

Fig. 7. Multiple shoot induction from germinated mature seeds of *S. album*

CONCLUSION:

Investigations of micro-propagation through the help of callus induction technique for *Sanctum album L*. were carried out.It was also observed that half MS medium along with addition of NAA was optimum medium or the seed germination. The in-vivo regeneration shows encouraging results for preservation of sandalwood whose presence is moving towards extinction.

REFERENCES:

1. Da Silva JA, Kher MM, Soner D, Page T, Zhang X, Nataraj M, Ma G. Sandalwood: basic biology, tissue culture, and genetic transformation. Planta. 2016 Apr 1;243(4):847-87.
2. Chang C, Chang WC. Micropropagation of Cymbidium ensifolium var. Misericors through callus-derived rhizomes. In Vitro Cellular & Developmental Biology-Plant. Nov 1, 2000 ;36(6):517-20.
3. Moon HK, Kim YW, Lee JS, Choi YE. Micropropagation of Kalopanax pictus tree via somatic embryogenesis. In Vitro Cellular & Developmental Biology-Plant. May 1, 2005;41(3):303-6.
4. Saeed W, Naseem S, Gohar D, Ali Z. Efficient and reproducible somatic embryogenesis and micro propagation in tomato via novel structures-Rhizoid Tubers. Bio Rxiv. Jan 1, 2018; 446286.
5. Bayarmaa GA, Lee NN, Kang HD, Oyuntsetseg B, Moon HK. Micro propagation of the Mongolian medicinal plant Zygophyllum potaninii via somatic embryogenesis. Plant Biotechnology Reports. Jun 2018;12:187-94.
6. Bakhtiar Z, Mirjalili MH, Sonboli A. In vitro callus induction and micro propagation of Thymus persicus (Lamiaceae), an endangered medicinal plant. Crop Breeding and Applied Biotechnology. Mar 2016;16(1):48-54.
7. Singh CK, Raj SR, Jaiswal PS, Patil VR, Punwar BS, Chavda JC, Subhash N. Effect of plant growth regulators on in vitro plant regeneration of sandalwood (Santalum album L.) via organogenesis. Agroforestry systems. Apr 1, 2016;90(2):281-8.
8. Al-Khayri JM, Naik PM. Date palm micropropagation: advances and applications. Ciência e Agrotecnologia. Jul 2017;41(4):347-58.
9. Divakaran M, Babu KN, Ravindran PN, Peter KV, Plant AJ. Biotechnology for micro propagation and enhancing variations in Vanilla. Asian J. Plant Sci. Res. 2015;5(2):52-62.
10. Mazri MA, Meziani R. Micropropagation of date palm: a review. Cell Dev Biol. 2015;4(3):160.

11. Shiji PC, Siril EA. An improved micropropagation and ex vitro rooting of a commercially important crop Henna (Lawsonia inermis L.). Physiology and Molecular Biology of Plants. 2018 Nov 1;24(6):1273-84.
12. Kaur A, Malhotra PK, Manchanda P, Gosal SS. Micropropagation and Somatic Embryogenesis in Sugarcane. In Biotechnologies of Crop Improvement,. Springer, Cham. 2018; 1: 57-91
13. Anandan R, Deenathayalan T, Kumar NS, Deepak KV. An alternative in vitro plant regeneration system in papaya (Carica papaya L.) through callus derived nodular cultures. Meta Gene. Sep 1, 2018 ;17:147-52.