SYNOPSIS OR OUTLINE OF RESEARCH ON (14Font)

"IN- VITRO SHOOT REGENERATION OF MARKING NUT (*Semecarpus anacardium L)* BY USING TDZ *"* (16 Font)

Submitted by (TNR 16Font)

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CONTENTS

CHAPTER- I (Font 16)

INTRODUCTION (Font 16)

(TNR Font 12) The plant *Semecarpus anacardium L* belongs to the family Anacardiacae. It is commonly known as Bhallataka, Bibba, Bhilawa, Dhobi nut and also marking nut (Krantikumar *et al.* 2015). It is a moderate size deciduous tree found in outer Himalayas and hotter part of India up to the 3500 ft height. The plant is found in abundance in Assam, Bihar, Bengal, Maharashtra, Orissa, Central India and also found in Western Peninsula and Australia. (Semalty, *et al.* 1983). It is also found in areas of Marathwada like Beed, some areas of Aurangabad, Parbhani, Hingoli and Nanded districts (Deshmukh *et al*. 2012).

It is medium sizes 15-25m height flakes. Leaves are simple, rounded at the apex, coriaceous, glabrous. Flowers are greenish in colours (Warrier *et. al*. 1996). The fruits are avoid, cup shaped, smooth, fleshy orange red in colour. It is attached with lower base thick out cover containing, shining weighs 3.5g. The outer and inner membrane of nuts contains corrosive resinous juice (Gauthaman *et al*. 2008). The extracts of seeds contain several alkaloids, flavonoids, bioflavonoids and other compounds (Rao and Row, 1973). Kernal of the nut contains 20-25% sweet, semidry non eadible oil (BSI 2004). The kernel of oil contains oleic acid- 60.6%, linolenic acid- 17.1%, palmatic acid-16%, stearic acid -3.8%, arachidic acid- 1-4%, (Jain Paras *et al. 2013*). The nutritive value of marking nut kernel for 100g (moister-3.8gm, Proteins-26.4 gm, fat-36.4gm, fiber-1.4, minerals-3.6gm, minerals- 3.6gm, carbohydrate- 28.4gm (Gouthman T. *et al.* 2008). That kernel of nut also contains 20-25% sweet, semidry non-eadible oil (BSI 2004).

Marking nut is one of most popular medicinal valuable plant in world of Ayurveda. Charak, Sushrut and Vagbhatt, the main three treatise of Ayurveda have described the medicinal properties and its formulation. Marking nut is classified in Ayurveda under the category of toxic plants. It is extremely hot and sharp in its attributes, it should be used with caution. Marking nut is a potent source of medicinal compounds prescribed for variety of ailments like leprosy, rheumatoid, arthritis, piles, asthma, cough, neurological disorders, skin diseases and sexually transmitted diseases such as syphilis and gonorrhea (Nandakarni, 1976). Two compounds, 1-2-dihydroxy-3-pentadecaenyl-8-benzene and 1,2-dihydroxy-3-benzene isolated from seeds of

marking nut demonstrated cyto-toxicity against human cancer cell lines (Shin *et al.* 1999). Due to toxic activities, large size, allergic effect and loss of traditional knowledge, generation by generation. Most of the people do not know the importance of marking nut. Marking nut use widely and gives economic returns also. The stem yields by tapping, an acrid, viscid juice from which a warnish is prepared. The nut yields a powerful and a substitute for marking ink for clothes by washerman. Hence it is frequently called as Dhobi Nut. It gives a black colour to cotton fabrics, but before application it must be mix with lime as a fixator. The fruits are also used as a dye. They are stringent, heat generating, appetizer, digesting, rejuventive, aphrodisiac herb and alleviates the skin and rheumatic disorders (Gouthman T. *et al*. 2008).

This plant included in endangered taxa (Mulchand *et al.* 2005), therefore it is need to develop culture practice for conservation. At present, it is not cultivated purposefully because it is found in forest region. The germination percentage of marking nut is 30% in soil at the month of December when it becomes fully mature. The germination percentage decreases by storing seeds for long time. Increase the germination percentage by braking dormancy and by using various plant growth regulators under *in-vitro* culture (Panda Mohan *et al.* 2009). Hard seedness is an adaptation allowing periods before inhibiting water and germination factor preventing more percentage of germination. Thidiazuron (TDZ), a substituted phenylurea (N-phenyl1,2,3-thiadiazol-5-yl-urea), is a potent bioregulator of *In-vitro* morphogenesis (Lu, 1993; Huetteman and Preece, 1993). It induces shoot organogenesis in several species of recalcitrant woody plants, and somatic embryogenesis in geranium, peanut, and neem (Murthy *et al.* 1998). Caulogenesis and somatic embryogenesis have been induced simultaneously in white ash (Bates *et al*. 1992) and chickpea (Murthy *et al.* 1996) using TDZ. TDZ stimulates the biosynthesis of cytokinins, which leads to an increase in the level of naturally occurring cytokinins (Ruzic and Vujovic 2008).

It is not cultivated purposefully because it is found in forest region. This plant included in endangered taxa, therefore it is need to develop culture practice for conservation and full fill the demand of market. TDZ is known that stimulates the biosynthesis of cytokinin and substituted phenyl urea is a potent bio-regulator of *In- vitro* morphogenesis. It induces shoot organogenesis in several species of woody plants. The present project research work will be carried out experiments conducted to overcome these limitations using TDZ of various concentrations

(5,6,7,8,9,10,11and 12 µM/lit) and standardization of protocol to achieve rapid, uniform, shoot regeneration of marking nut under *In- vitro* condition.

OBJECTIVES (TNR FONT 16)

The WPM medium with addition of thidiazuron (TDZ) will be used as treatments to investigate shoot regeneration of marking nut (*Semecarpus anacardium L.*). The project research work will be conducted with following objectives.

- To find out optimum dose of TDZ for shoot induction in *Semecarpus anacardium L*.
- Effect of different concentrations of TDZ on cotyledonary nodal explants.

CHAPTER- II(TNR FONT 16)

REVIEW OF LITERATURE (TNR FONT 16)

An attempted has been made in this chapter to review the research work done in past on the aspect of present by various scientists in India and Abroad.

Lloyd, G. and Mc. Cown, (1980) formulated McCown woody plant medium for *In-vitro* propogation of woody plant species with different concentration of growth regulators.

Chakraborty, S. , Roy, M., *et al.* (2004) studied the cytotoxic effect of root extract of *Tiliacora racemos* and oil of marking nut in human tumor cells. He used four human tumor cell lines: acute myeloblastic leukaemia (HL-60), chronic mylogenic leukemia (K-562), breast adenocarcinoma (MCF-7) and cervical epithelial carcinoma. The cells were grown in appropriate media. The ethanol extract of *T. racemosa* root and marking nut oil prepared according to the Ayurveda principle were found a cytotoxic activity. These alkaloid fraction extracted from *T. recemosa* had maximum cytotoxicity and was effective against all four cell lines and Marking nut oil was cytotoxic only in leukemic cells.

BSI, (2004) Kernal of the nut contains 20-25% sweet, semidry non eadible oil (BSI 2004). The kernel of oil contains oleic acid- 60.6%, linolenic acid- 17.1%, palmatic acid-16%, stearic acid -3.8%, arachidic acid- 1-4%, (Jain Paras *et al. 2013*). The nutritive value of marking nut kernel for 100g (moister-3.8gm, Proteins-26.4 gm, fat-36.4gm, fiber-1.4, minerals-3.6gm, minerals- 3.6gm, carbohydrate- 28.4gm (Gouthman T. *et al.* 2008). That kernel of nut also contains 20-25% sweet, semidry non-eadible oil.

Bhuban, and Hazara (2009), reported the germination percentage of marking nuts is 30% in soil. It decrease after storing for long time. He also explained the 98.8% H2SO4 concentrated treatment to the seeds for 20 minute it increases germination percentage upto 70%.

Aseervatham, J., Palanivels, S., and Sachdanandam, P. (2010) performed an experiment on rats and proved that cyto-protective effect of marking nut against toxicity induced by *Streptozotocin* in rats. He got a result that marking nut able to reverse the level of the marker enzymes and protect the kidney by reverting back to the normal levels of urea, uric acid and creatinine.

Panda, *et al.* (2010), developed a method of proliferation of shoots from axillary meristem of marking nut was achieved in semisolid WPM medium supplemented with BAP 4.44uM and kinetin 4.64 µM concentrated of cytokinin hormones.

Bhuban *et al*. (2011), were noted three different morpho-genic responses caulogenesis, direct somatic embryogenesis and callusing in cotyledonary explants of marking nut containing various concentration of thidiazuron (TDZ). He found that 9.08 µM/L concentration of TDZ induced best shoot among the media.

Jain Paras and Sharma, H.P. (2013), observed that the extract of marking nut seeds are used by traditional physicians and healers in their clinical practice. They have done some experiment and proved marking nuts anti-atherogenic, anti-inflammatory, antioxidant, antimicrobial, anti-reproductive, CNS-stimulant, hypoglycemic, anti-carcinogenic and hair growth promoter activities.

Jain Paras *et al*. (2014) proved that the different concentration of methanolic extract (6.25, 12.5, 25, 37.5, 50, 62.5 ug/ml) of marking nut were tested against four fungal strains namely *Fusrium oxysporium, Rhizoctonia Solanii, Alternaria* species and *Sclerotium rolfsii*. He observed excellent inhibitory activity was observed against *Rhizoctonis solanii* (100%) followed by *Sclerotium rofsii* (92.59%) *Altrnaria spp*. (72.34%) and *Fusarium oxysporum* (47.19%) at 62.5µg/ml. Among different fungi tested *R. Solanii* and *Sclerotium rolfsii* were found to be more sensitive to crude extract when composed to others.

Krantikumar Amaley, and Jain Atul, (2015) proved that semecarol (monohydroxy phenol), and shilawanol O-dihydroxy (compound) is the active constituent in marking nut. Has been found to be large responsible for the therapeutic potentials anti-helminthis, cardiac stimulant etc.

CHAPTER- III (TNR FONT 16)

MATERIALS AND METHODS (TNR FONT 16)

The details of various material and methods will be conducting during the course of present investigation are narrated in this chapter under suitable sub-heads.

Source of plant material and Experimental site: The seeds of marking nuts will be collected from the Forest Department, Buldana and used as an experimental material. The experiment will be conducted in Department of Plant Biotechnology MGM College of Agricultural Biotechnology, Gandheli, Aurangabad during 2016-17.

Details of TDZ at different concentrations: The WPM medium will be supplemented with different concentrations of TDZ (5 6, 7,8,9,10,11 and 12 µM/L) for the induction of shoot in marking nut through cotyledonary nodal explants. The details of statistical design will be applied for above said objectives.

Statistical design **:** Completely Randomized Design (CRD).

No. of treatments **:** 08

No. of replications **:** 03

Treatment details **:** Concentrations of TDZ

Treatments (T)	Concentration of TDZ (µM/L)
T_1	5
T_2	6
T_3	7
T_4	8
T_5	9
T_6	10
T_7	11
T8	12

***Note- WPM (20ml) constant from T_1 to T_8**

Explant preparation (Seed): (TNR FONT 14) Mature marking nuts will be collected during December from trees growing naturally. Wash the seeds thoroughly under running tap water and remove the pericarp and mesocarp layer of marking nuts manually. The nuts will soak in concentrated H_2SO_4 for 20 minutes respectively. The treated seeds will keep at cold condition

(4^0C) for seven days. Then, seeds will be treat with 2% bavistin. At the end seeds will treat with 0.1% Sodium hypochloride solution and sterile water. Transfer these treated seeds into the WPM medium for germination.

Explant preparation (Nodal): Nodal explant of marking nut will be collected from Himayatbag, Aurangabad. The nodal explants will be washed with tap water for one hour and then with 2 drops of Tween-20 and 2-3 drops of laboline respectively. Followed by surface sterilization of explants by using 1% sodium hypochlorite and 70% ethanol. Wash the explant with three to four times by using sterile distilled water to remove the precipitate of sodium hypochlorite. All the procedure carried out under laminar air flow.

Preparation of shoot induction media (WPM MEDIA): The WPM (woody plant medium) medium supplemented with various combinations of Thidiazuron (TDZ) will be used for shoot initiation containing $CaCl_2$, vitamin and sucrose. The pH 5.6-5.8 will be adjusted to 1N NaOH or 1N HCl and volume will be made up by sterile water. Then agar (0.7%) will be added and 2 to 3 drops of antifungal and antimicrobial supplement. After that the media will be uniformly mixed with magnetic stirrer and autoclaved at 121ºC for 20 min. The sterile WPM medium will be supplemented with different concentrations of TDZ (5, 6,7,8,9,10,11,12 µM/L) by using syringe filter (0.22µM).

Inoculation (Seed): In each test tube, WPM medium (20 ml) will be supplemented with different concentrations of TDZ ranging from 5 µM/L to 12 µM/L for the induction of shoot from cotyledon nodal explant. The treated seeds will be inoculated into test tube containing WPM media for aseptic seed grow.

Inoculation (Nodal): In each test tube, WPM medium (20 ml) will be supplemented with different concentrations of TDZ ranging from 5 µM/L to 12 µM/L for the induction of shoot from cotyledon nodal explant. The treated nodal explants will be inoculated into test tube containing WPM media.

Incubation: Cultures will be maintained at 2000 to 3000 Lux light density with photoperiod of 16 hour provided by cool white fluorescent. This photoperiod will be maintained at 16 hours light and 8 hours dark with relative humidity 60-70%. For better shoot induction, the culture will be kept in dark at 25°C (±1°C) and 70% relative humidity. The result will occur after 35-40

days. The fresh shoot size will be recorded and the significant difference will be analyzed by Analysis of Variance (ANOVA).

Biometric observations:

1. **Height of explants:** The shoot length will be measured from base to tip of the plantlet at the time of inoculation and the average length will be express in centimeters.
2. **Days required for shoot initiation:** The numbers of days take to show initial differentiation of shoot from the date of inoculation (DAI) of different explants will be record and will be as mean number of days.
3. **Number of leaves:** The number of leaves proliferate will be measured, after inoculation will be record.

Analysis of Data: The experiment will be conducted under completely randomized design (CRD). The data obtained on various observations will be analyzed by "Analysis of variance" method (Panse and Sukhatme 1967).

CHAPTER- IV (TNR FONT 16)

Scope of the Project

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Signature of student

Research Project Guide

Dr. S.N.Harke

Department of Biotechnology Submitted a copy of Outline of Research Work (ORW) in triplicate, to the Degree Coordinator, Chairman and Project Guide for approval of Research Project Work(HOT 481).

Chairman

Director

Approved/ Not Approved

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