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MICROPROPAGATION OF MATURE *TERMINALIA CATAPPA* VARIETIES

Forestry Science

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ABSTRACT

Micropropogation is used to multiply plants such as those that been genetically conventional plant breeding with the multiplying progeny plants. Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micropropagation. The success of Plant tissue culture in recent years approaches have been used as an efficient tool for micropropagation of trees in short time. [1][2]**.** Propagation of woody trees through tissue culture has many advantages over conventional propagation with different methods like fast growing, season independent of production of the plant, germ plasm conservation and facilitating their growth. This review provides an overview of the success achieved on in vivo work done for a number of important forest trees.Rapid culturing of producing clonal plants this review states that invitro work has been done for no of important forest trees **[3].** Both the varieties were subjected, nodal explants were subjected with MS Media and Growth hormones with the dilution of kinetin with 1-1.5 mg/ml and with the mixture of both Kinetin, BAP with the dilution of 1-3 mg/ml with an increase of time for 3-5 weeks the growth was 3-6 cm length. The study of micropropagation in both the varieties, all the values of the work are Mean of at least 3 independent experiment. The MEAN±SEM and the extract number of experiments are given in legends. The Significance of difference between control and each trial treatment was analysed using ANOVA showed statistical results as and ANOVA is $p \leq 0.05$*is significant.

KEYWORDS

Terminalia catappa, Micropropogation, Nodal culture, BAP, Kinetin.

1.INTRODUCTION:

Botanical derived medicinal plants play a major role in human society [4]. Plant extract has active ingredients [5] and extracts from the extraction of the medicinal plants contain several phytochemicals that can work alone or synergistically with others against various ailments.

Terminalia catappa is an ornamental tropical tree belonging to the Family Combretaceae native to Southeast Asia in tropical regions of Asia, Africa and Australia. *Terminalia catappa* L has larger, glossy, dark green leaves, thicker branchlets, longer flower spikes and larger fruits. It is known by the common names such as Bengal almond, Country almond, False kamani, Indian almond, Malabar almond, Sea almond and Tropical almond [6]. The roots, stems, bark, leaves and fruits of these medicinal plants play an integral part in tackling diseases. The edible fruits from some of these plants are both nutrition, medicinally, nutritionally as well as pharmaceutically to human being. This plant is originally from Africa, Asia and Australia before spreading to other parts of the world. Due to globalization green revolution. It also causes significant litter on the ground [7]. *Terminalia catappa* featured high content of nutrients and polyphenols needed for biological metabolism and human health. In addition, heavy metals were also present at traces level indicating that these plants would be safe for medicinal uses [8]. Tropical almond can be consumed or used for preparing fruit salad, smoothie or for garnishing dishes. Oil can be extracted from the dried nuts which can be used for cooking. *Terminalia catappa L* is a Combretaceous plant, the extract of leaves and bark are reported for their anticancer, anti-HIV reverse transcripts, hepato-protective, anti-inflammatory, hepatitis, antidiabetic, as well as aphrodisiac. The leaves are used as food for the Tasar silkworm. The therapeutic potential of this plant is a primary importance to analyse its phytochemicals, secondary metabolites, antioxidant and antimicrobial activities, as it is vital in everyman's life throughout the history due to its usefulness either in the form of raw or processed or combined to drug find's application in pharmaceutical industries for health care [9].The present study of the medicinal plant has been investigated in various pharmaceutical studies as it contains a variety of chemical component [10]

Micropropagation is the practice of rapidly multiplying stock plant material to produce and large number of progeny plants using modern plant tissue culture methods, an extremely effective tool. It is artificial process of producing vegetatively through tissue culture or cell culture techniques. This method is a production of cells from meristematic culture, callus culture, suspension culture, and embryo culture protoplast culture. A wide range of fruit ornamental and forest species are currently interested as for invitro propogation. The developed regeneration system is also used to developed regeneration system are also used to optimize effective cryopreservation technique. The most widely used application of the tissue culture technology in agriculture and forestry. Plant tissue culture is a technique that has been as a technology around or more than 30 yrs. This process is an important technology in developing countries for the disease-free high-quality planting material. This technique is used to produce large number of identical plants (clones) from a selected stock plant, propogation of rare species. Propogation of plants can be achieved through three regeneration pathways that is axillary bud proliferation, somatic embryogenesis and adventitious bud formation [2,3]. The history of plant tissue culture and its applications have been reviewed and discussed.

Micropropagation is conventional plant breeding method; multiplication is the taking of tissue samples produced during the first stage and increasing their number. Following methods are the successful introduction and growth of plant tissue invitro. Plants can be propagated by sexual or asexual means clonal propagation refers to the process of asexual reproduction by multiplication of genetically identical copies of individual plants. Clone is used to represent a plant population derived from a single individual by asexual production. It is faster multiplication, large number of plants can be produced from a single individual in a short period possible to produce genetically identical plants. In this process sterile hybrids can be easily propagated in plants, tendrils. This method is expensive frequently unsuccessful. A handy technique for rapid multiplication of plants. Plant regeneration processes are called as organogenesis and somatic embryogenesis, artificial seed propogation, study of genetic variability, production of disease-free plants. Totipotency of the cell has the genetic makeup capable of developing a pathway leading to the formation of an entire plant that is identical to the plant from which it is desired under the control of the invitro of high intensity-controlled temperature and a defined nutrient medium. This technique is used to establish and maintain virus–free plant stock, it is one of the conventional methods in the aseptic condition are essential to achieve success. It is a commercially vegetatively propagated plant species. A tissue culture is a technique used for plant propagation in which a part of tissue is taken from plant and grown in a laboratory to produce plantlets that are genetically identical to parents. National council of Italy said the identical cultures are identical to the parent. It is an Innovative technique of high importance in Italy 11 Recent studies on medicinal plants are the most important source of life saving drugs for the world population showed that the culture stimulates the development of axillary buds, apical dominance which allows massively the elongation of shoots in gelled media. Objective of production for virus free stock, multiply plants, produce progenies which are genetically

identical to their parents, genetic variability and recovery of distant hybrids, germplasm conservation and exchange of genetic transformation. Conservation of germplasm and also for propagation of a sustainable utilization of forest trees is a major application of plant tissue culture. Micropropagation of tree species offers a rapid means of producing clonal planting stock of afforestation woody biomass production and conservation of elite and rare germplasm. This review provides an overview of the success achieved on in work done for a number of important forest trees. In recent years use of tissue culture technique for clonal propagation of forest trees has increased considerably by using juvenile as well as mature plant parts as starting material many tree species have been propagated successfully through invitro shoot proliferation from cotyledonary nodal explant of seedlings.

Micropropagation are being used by increasing number of research work and commercial firms. Tremendous progress in plant tissue culture resulting in advances, successful and influenced by a variety of factors that are categorized either as environmental or hormonal-plant growth regulators factors, acclimatization and subsequent standard method of propagation for many species of economic importance and mass production scaled mechanized efficient and integrated systems of micropropagation will allow mass production of important in vitro derived products as flavorings, pharmaceuticals, health beneficial plant compounds. Sufficient literature is available on various aspects of tissue culture studies in important plant and desert trees. As an application of Micropropagation, the Plant tissue culture is sufficient for the production of millions of clones in a year using micropropagation. It would be great deal of time to produce an equal number of plants using conventional method has more scope and future. This technique of micropropagation is a good alternative for those plant species that show resistance to practice of conventional bulk propagation. It is an alternative method of vegetative propagation in a short period. Any particular variety may be produced in large quantities and time to develop new varieties is reduced by 50% large number of plants are maintained in small spaces and storage of germplasm. Production of in vitro stock can be done at any time of the year. This technique is useful for seed production in certain crops as the requirement of genetic conservation to a high degree is important for seed production. Somatic embryogenesis is the production of synthetic artificial seeds.

2.1Review of literature:

The main objective of tissue culture work was ascertaining the possibility of culturing the cell indefinitely. Plant biotechnology study reveals that having tissue culture as an important tool offers approaches for genetic improvement of any species. Micropropagation is a powerful tool for fast multiplication of selected genotypes at faster rates; therefore, it has been adopted in agriculture as well as in forestry. *An innovative approach to tissue culture using meristematic cells like root and stem tips was reported [11]. A Significance breakthrough in tissue culture was the discovery of Auxin* [12]. Revised medium for rapid growth and bio-assays with tobacco tissue cultures is studied. Physiology of the Plant was conducted [2].

The history of plant tissue culture begins independently stated the cell theory and proposed the totipotency which states that the cells are autonomic in principle are capable of regenerating to give a complete plant. Invitro vegetative propagation methods were started with Ball 1946 pointed out exactly which part of a shoot meristem gave rise to a whole plant. Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micropropagation, the success of plant tissue culture [1]. *Several predictions about the requirements in media in experimental conditions which induce cell division proliferation and embryo induction and he is regarded as father of tissue culture [13].*

Depends on the choice of the nutrient as required by the whole plant, growing in vitro are mainly heterotropic, that is they cannot synthesize their own food as mass values of providing them with the macronutrients and micronutrients ($\mu mol/l^{-1}$). Plants containing beneficial and medicinal properties have been known and used as sources of food, fodder, oils, medicines fuel, wood, fibres and timber by increasing population growth due to increased demand for pulp, paper, construction materials, farmlands and fuel, status of woody trees especially forest trees are greatly affected. Plant is our wealth and its conservation is important for economic ecological and scientific medicinal and ethical issues therefore, there is a great need to conserve forest ecosystem by agrotechnology in recent years approaches have been used as an efficient tool for micropropagation of trees in short time. Propagation of woody trees through tissue culture has many advantages over conventional propagation with different methods like fast growing season independent of production of the plant germ plasm conservation and facilitating their growth.

Micropropogation of tree species offers rapid means of producing clonal plants this review states that invitro work done for number of important forest trees.[3] Cotyledenary node explants excised from 21-day old seedlings of *T.arjuna* produced multiple shoots were cultured on full strength of modified MS (1/2MS and Fe-EDTA). Proliferating shoot culture was established by reculturing the original cotyledonary nodes 88% shoots could be obtained well after 15hrs of pulse treatment with IBA[15]. The cotyledonary explants excised from 2-day old seedlings of *Terminalia arjuna* produced multiple shoots when cultured on full strength. MS or modified M S Media with different concentration (0.1-1.0 mg/ml) of BAP maximum [4][7]. Shoots explants were recorded after 30 days of inoculants about 80% of the plantlet were successfully acclimatized [14]. The different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation [1516,17].

Micropropagation of mature *Terminalia catappa* (Indian Almond) a medicinally important forest tree reported on efficient invitro propagation of *Terminalia catappa* using nodal segments of a 15-year-old mature tree nearly 75% of the plantlet could be acclimatized within 5 weeks and successfully established this is the first report on micropropagation of *Terminalia catappa* which can be applied for further genetic transformation assays and pharmaceutical pourposes [2].

An efficient and improved invitro propagation method has been developed for *Terminalia bellarica* a medicinally important tree from nodal explants of 10 years old mature tree. Shoot multiplication was influenced successive transfer of mother explants for differentiation and subculture with excised shoot on fresh medium of MS Medium containing 2.22µM BAP was found to be the shoot multiplication. Further enhancement in morphogenetic response when excised shoot clumps on MS medium supplemented with 2.22 µM BAP, 1.16 µM Kn and 0.5 µM IAA. Plantlets rooted in vitro as well as exvitro were acclimatized successfully under the green house condition. Plant production serves as a more economical option present method is used for large scale commercial production of this medicinally important tree. The trial investigation to the effects of coconut milk on stem and root cuttings of *Terminalia catappa* there was a significant interaction between cutting type and coconut milk should be used for vegetative propagation programs of species. Rapid invitro micropropagation protocol of *Terminalia bellarica* was achieved by nodal explants when placed on medium supplemented with 0.5mg/l, 6-BAP showed 100% shoot bud with 4.5-0.56 shoot length per explants. The nodal segments from micro shoot obtain from induction medium were cultured on MS medium supplemented with different concentration of hormones like BAP, NAA. These cultures were placed in green house for primary hardening. After four weeks plants were transferred from green house to net house where the plants exhibited gradual acclimatization to outdoor conditions. Nodal segments obtained from 15 day old aseptically grown seedlings were used as explants, shoot multiplication was achieved on MS medium containing BAP and Kn. The maximum number of shoots was obtained with 3.5 mg/l BAP +0.5 mg/ L Kn. Best rooting was observed on medium containing quarter strength MS salts, 0.8% agar and 1.0 mg/L IBA. Plantlets were hardened initially in culture room conditions and then transfers to mist house. Maximum callus induction response was observed on M S medium supplemented with 0.25 mg/l, 2, 4-D + 0.3 mg/L Kinetin within 4 weeks from leaf petiole. Maximum callus induction response was observed on medium supplemented with 0.25 mg/ml of 2,4D +0.3 mg/ml of kinetin within 4 weeks from leaf petiole.

The result of this study in the leaf architectural characters are of great help in plant taxonomy and systematic most especially in dealing with sterile plant specimens19. The micropropagation of *Terminalia bellarica* from mature tree M S medium gave best shoot bud proliferation system in incorporation of antioxidants and PVP, the mother plant confirmed true type clonal explants. The study reported that *Terminalia bellarica* from nodal segments of a 30 years old tree.

Nodal segments taken from the mature tree in month–April and cultured on half strength reported MS medium gave the best shoot bud proliferation response. Combinations of series transfer technique (ST) and incorporation of antioxidants (AO) (Polyvinylpyrrolidone, PVP (50 mg/L) + ascorbic acid (100mg/L) + citric acid (10 mg/L).

A reliable and efficient micropropagation protocol was developed through axillary shoot proliferation from nodal explants of mature *Terminalia arjuna*. Nodal segments of fresh sprout were used as explants produced optimum number of shoots through activation of axillary buds on modified MS medium, maximum (100%) invitro shoots proliferation was obtained. Invitro multiplication of plant in M S Medium was used for maximum 100 mg/L activated charcoal. Invitro multiplication was obtained with 8.86µM BAP and additives, this method was used for large scale commercial production of medicinally important tree. A protocol for micropropagation of plants via axillary bud proliferation from nodal explants *Terminalia bellarica* seedlings has been established. Explants were cultured on MS media with different concentration of 6-benzyladenine hormone and kinetin and then separated and transferred to fresh medium with the lower levels of BAP on Kn. Rooting of the shoots was achieved under the invitro condition on two different media was tested like Gamborgs medium and woody plant medium. Regenerated plants were established in the greenhouse, Regeneration occurred via organogenesis and embryogenesis in response to auxins and cytokinin's. The integrated approaches of our culture systems will provide the basis for the future development of novel, safe, effective and high-quality product of consumers [16].,

3. MATERIAL AND METHODS:

Selection of plant material: Nodal explants material were collected from the mature tree for two varieties. The collected material was checked free from diseases. The material was excised with a scissor as explants and kept in petriplates taken into the culture lab.

3.1.1. Disinfection of the plant: The explants was washed in running tap water for about 30 min. Then drop of liquid soap tween 20 was added into the flask and explants were agitated.

3.1.2. Pre-treatment of culture: The nodal cultures are treated with the Tween 20/1 % and 0.1% $HgCl_2$ solutions as pre-treatment for sterilization.

3.1.3. Preparation of explants: Nodal explants after cleaning with disinfection were cut into the small pieces of about 1 cm consisting of a size axillary bud and placed in a conical flask.The size of the explants play a key role in experiment of the morphogenetic potentially and small explants are good for initiation.

3.1.4. MAINTENANACE OF ASEPTIC CONDITION IN THE INCUBATION CHAMBER

Cleaning of glassware Different types of glassware's used to culture the plantlets invitro. Borosilicate glass was used to culture the plant tissue. Glassware was washed with soap oil, autoclaved at particular temperature and transferred to hot air oven in 1L conical flask. All the media and glassware's were taken to the LAF which was also cleaned with 70% ethanol and chamber from contamination. After 20 min airflow was switched on along with UV lamp to blow away all the dust etc. This is the method to clean the chamber as aseptic condition were a prerequisite for the culture.

3.1.5. Media sterilization: The media contains nutrients for growth of explants which are rich in sucrose and other organic nutrients support the growth and maintain complete aseptic environment inside the culture vessels.

3.1.6. Media Evaluation: Screening of different basal media that is MS Media was carried out for the establishment of explants. The composition of the basal media is furnished with required nutrients.

3.7. Growth Hormones: Plant growth regulators were used in combination with media these solutions are thermolabile cannot be autoclaved they are sterilized by membrane filtration and added to the autoclaved medium and cooled.

3.8. Pre-Inoculation activities:

The plant material node to be inoculated was placed inside the chamber. The hands and figures were swiped with rectified spirit. The explants in the flask were added with 0.05% to 1% Mercuric chloride and gently agitated from time to time for 2 min-3 min depends on the explants. The inoculation forceps kept rectified spirit in coupling jar and was brought the flame and muslin covered cotton plug. The explants in petridish were carefully transferred to the test tube and implanting the mouth of the test tubes flamed and plugged. The cultures were incubated at 2000 luminous lights under cool white fluorescent tubes. The cultures were maintained at approximately at 26±2°C.

3.2. Procedure of inoculation: The plant tissue culture lab is sterilized with the fumigation and the cleaned with 70% ethanol and the explants is taken in sterile petriplate and excised into small pieces with the help of sterile forceps and needle the explants were then transferred carefully and aseptically into test tube culture bottle with M.S. Media with the hormone dilutions.

3.2.1. Incubation of culture: A constant ambient temperature of 25+2°C maintained in the incubation room (culture room) under controlled condition.

3.2.2.Constituents of Media: Many elements are needed for plant nutrition and their physiological functions; thus, these elements have to be supplied in the culture medium to support adequate growth of cultures invitro although they require in minute quantities these include Iron, Magnesium, Zinc, Molybdenum[2]

3.2.3. Carbon and energy source: Plant cells and tissues in the culture medium are heterotropic and therefore dependent on external carbon source of energy during the course of sterilization of the medium, sucrose gets hydrolysed to glucose and fructose.

3.2.4. Organic supplements: The organic supplements include vitamins, aminoacids, organic acids, organic extracts activated charcoal and antibiotics.

3.2.5. Vitamins: The vitamins added to the medium to achieve goal growth of cells the vitamins to achieve good growth of cells. The vitamins added to the medium should be supplemented with vitamins to achieve growth of cells which include thiamine, riboflavin, niacin.

3.2.6. Aminoacids: Although the cultured plant cells can synthesize aminoacids to a certain extent media supplemented to stimulate cell growth and help in establishment of cell lines.

3.2.7. Organic acids: Citrate, succinate, fumarate allow the growth of plant cells, Pyruvate also enhances the growth of plant cells.

3.2.8. Activated charcoal: Supplementation of the medium with activated charcoal stimulates the growth differentiation of certain plant cells, facilitates efficient cell growth in cultures and phytohormones.

3.2.9. Growth regulators: Plant hormones or phytohormones are group of cultural organic compound that promote growth development and differentiation of plants. Four classes of plant hormones are like Auxin, Cytokinin, Gibberellins, Abscisic acid helps in organogenesis and differentiation.

3.2.10. Auxins: They induce cell division, cell elongation and formation of callus in cultures. At a low concentration of auxin promote root formation while at a high concentration callus formation.

3.2.11. Cytokinins: The group of plant hormones that promote cell division and retard aging in plants they are also called as kinetin they promote cell division, growth and delay senescence of leaves.

3.2.12. Gibberellins: The Growth hormones that stimulate cell elongation and cause plants to grow taller stem elongation, germination, flowering and fruit ripening.

3.3. Preparation of stock: Plant tissue require actively group part of plant, supply of continuous inorganic chemicals which constitute the macronutrients and micronutrients, potassium and ammonium nitrate, Potassium dihydrogen phosphate was used in combination for M S Medium.

3.3.1 Preparation of M S Media: M S Media concerned stock solution of macronutrient and micronutrient. Plant Growth Regulator

like auxin, Gibberellins, Cytokinin is the hormones frequently used in tissue culture work. Abscisic acid and ethylene are also used. Vitamins like Thiamine HCl, Nicotinic acid, pyridoxine HCl were added. Activated charcoal 0.1% was added as supplemented to the nutrient media as it adsorbs secondary products. Carbon source 20,000mg/l, sucrose was also added. Sterile double distill water is used for the preparation of media.

Micropropogation is a technique of plant tissue culture aseptically cultured in the plant tissue culture room. The explants were excised in to 1mm length sterilized with the help of sterilant. The MS media is prepared according to the concentration required and autoclaved at the temperature and pH value monitored as per standard values. The explants were subjected to sterilization with $Hgcl_2$ (0.1-1.0%) with the time limit of one minute and media is supplemented with the activated charcoal and citric acid to remove the phenolic contamination.

Table 3: Composition of M S Media with Growth hormones

4. Result and Discussion:

COMPOSITION	**Major salts (macronutrients) Table 1:Composition for M S Media**	M.S.Media
Macronutrients	Ammonium nitrate (NH_4NO_3)	1,650 mg/L
	Calcium chloride ($CaCl_2 \cdot 2 H_2O$)	440 mg/L
	Cupric sulphate ($CuSO_4 \cdot 5 H_2O$)	370 mg/L
	Potassium phosphate (KH_2PO_4)	170 mg/L
	Calcium nitrate	----------
	Ammonium sulphate	----------
	Potassium nitrate (KNO_3)	1,900 mg/L
Minorsalts (micronutrients)	Boric acid (H_3BO_3)	6.2 mg/L
	Cobalt chloride ($CoCl_2 \cdot 6H_2O$)	0.025 mg/L
	Cupric sulphate ($CuSO_4 \cdot 5H_2O$)	0.025 mg/L
	Ferrous sulphate ($FeSO_4 \cdot 7H_2O$)	27.8 mg/L
	Manganese sulphate ($MnSO_4 \cdot 4H_2O$)	22.3 mg/L
	Potassium iodide (KI)	0.83 mg/L
	Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$)	0.25 mg/L
	Potassium sulphate	----------
	Zinc sulphate ($ZnSO_4 \cdot 7H_2O$)	8.6 mg/L
	$Na_2EDTA \cdot 2H_2O$	37.2 mg/L
Vitamins and organics	Inositol	100 mg/L
	Niacin	0.5 mg/L
	Molybdic acid	0.25mg/L
	Nicotinic acid	----------
	EDTA	37.3
	Pyridoxine · Hcl	0.5 mg/L
	Thiamine · Hcl	2 mg/L
	Glycine	0.1 mg/L
	SUCROSE	30mg/L
	Agar	20mg/L
With the hormones	Kinetin, BAP	1-3mg/l

The explants were also sterilized are inoculated in the sterilized LAF chamber. The media composition was prepared with the proper addition of growth hormones like Kinetin and BAP with the dilution of 1-3 mg/ml.

In the first response the explants were inoculated for both the varieties T1 Yellow variety started response for growth initiation with MS media with BAP (1 to 1.5 mg/ml) and MS media with Kinetin so there is no growth in response later MS Media with the combination of Kinetin and BAP with the increase in the concentration of hormones from 1mg/ml to 2mg/ml and increase to 3mg/ml increases the growth only in *Terminalia catappa* T1Y, Yellow variety than Red variety. T2R, Red variety has phytochemically more concentration of phenol so there was contamination browning of the media so growth has stopped only Yellow variety continued till it reached the shoot length of 1cm to 4cm and increased to 5-6 cm. The growth curve increases as the growth medium supplements proper nutrients.

Micropropogation showed response in both the varieties with growth regulators for hormones Kinetin and BAP for 3 weeks and it is showing growth without fungal contamination. For initiation T1 variety MS medium with the kinetin, BAP hormone 2.0 mg/l showed growth for 3.0 mg/l of about 1cm to 6cm.T2 variety has shown no growth due to the phenolic compounds. The stages of growth in T1 variety have shown with full-fledged from 2- 3 months as in fig 1 to fig 9 and Table 1 and 2.

Table 1- M S Media composition with Growth Hormones

Sl. no.	MEDIA MS+KINETIN (Plant Hormone)	T1SHOOTS/ EXPLANTS	Dilutions	T2SHOOTS/ EXPLANTS	T1/T2 LENGTH cm /shoot
1	M S +BAP	GROWTH RESPONSE	1mg/l	No Response	----------
2	MS +BAP	GROWTH RESPONSE	1.5mg/l	No Response	----------
3	MS + KINETIN	GROWTH RESPONSE	1mg/l	No Response	----------
4	MS + KINETIN+ BAP	GROWTH RESPONSE	1.5mg/l	Initiated	1 cm
5	MS + KINETIN+ BAP	GROWTH RESPONSE	2mg/l	Further growth continued	4cm
6	MS + KINETIN+ BAP	GROWTH RESPONSE	3mg/l	Good response	5-6 cm

Table 2: Growth chart of Micropropogation for Yellow and Red varieties.

Sl. no.	Variety	Media	Hormones	Weeks	Response	Growth
1	T1(Yellow variety)	MS+KINETIN+BAP	1-3mg/l	5 weeks	Showed initiation	Growth started with initiation, showed full length growth curve from 3cm,5 cm and 6 cm.
2	T2(Red variety)	MS+KINETIN+BAP	1-3mg/l	No response	Showed initiation Brownish color is formed due to the presence of phenolic contamination.	Growth was not successful and stopped.

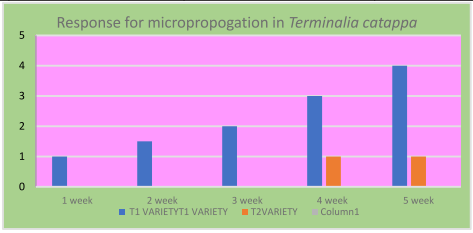

Fig 1: Graphical representation of Micropropagation

Fig 2: Invitro culture of T1Y and T2R Nodal explants initiation

Fig 3: Invitro culture T1Y and T2R Nodal explants 1st week

Fig 4: T1Y AND T2R Nodal cultures showed initiation in both the varieties 3 weeks

Fig 5: Growth of T1Y variety, stages for 2 months.

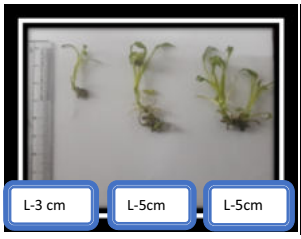

Fig 6: Measurement of explants for T Y Variety

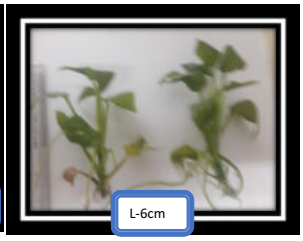

Fig 7: T1Y variety- full length growth for 3 months

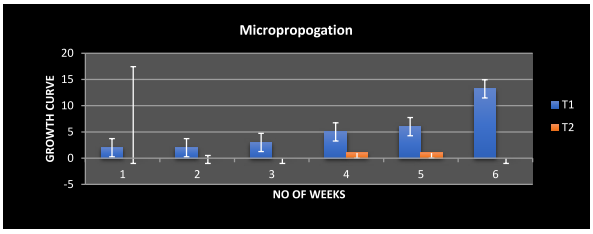

Fig 8: MEAN±SEM for Micropropogation

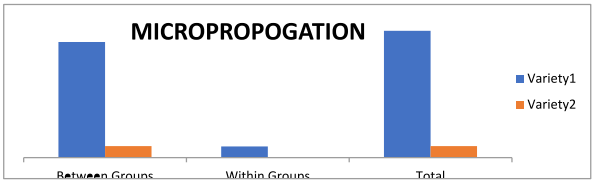

Fig 9: ANOVA results for Micropropogation

5. CONCLUSION:

Micropropagation is the method used for development of shoots, roots from explants and multiple subcultures. The present work described that the successful regeneration of *Terminalia catappa* through nodal culture. It is concluded that the nodal explant of *Terminalia catappa* T1Y required different level of hormones which helps in the growth of the plant than T2R and both the variety are grown invitro for its medicinal value. In recent years use of tissue culture technique for clonal propagation of forest trees has increased considerably by using juvenile as well as mature plant parts as starting material many tree species have been propagated successfully through invitro shoot proliferation from cotyledonary node explant of seedlings. Tissue culture has paved promising technique for conservation and multiplication of large-scale production of tree species to shorten timings, less cost and small area and regeneration of plantlets in nursery for further usage of medicinal properties. Growth Hormone BAP is best suitable which is used in plant tissue culture technique. The invitro technique is further used for molecular studies, genetic engineering, pharmaceutical properties. Micropropagation T1Y variety has the highest amount of bioactive compounds than compare to T2R variety.

6. STATISTICALANALYSIS:

The study of micropropagation in both the varieties, all the values of the work are Mean of at least 3 independent experiment. The MEAN±SEM and the extract number of experiments are given in legends. The Significance of difference between control and each trial treatment was analysed using ANOVA showed statistical results and ANOVA is $p \leq 0.05$* is significant.

7. Acknowledgement:

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8. REFERENCES:

1. Indra K., Vasil Trevor., Thorpe A., Plant cell tissue culture, Springer, Journal of Forest Research, 17:22, (1994).
2. Murashige T and Skoog F., (1962), A revised medium for rapid growth and bio-assays with tobacco tissue culture, *Physiol Plant*, 15(3): 473-497.
3. Kataria N.,Yadav K., Kumari S and N Singh ., Micropropogation ;An important tool for conserving forest trees, Pertanika J,Agric Sci,36(1):1-26,(2013).
4. Edward F and G.W. Dennis, Tropical almond (*Terminalia catappa*). A Series of the Publication of the Environmental Horticulture Department, *Florida Fact Sheet* ST626, (1964).
5. Nair R.,Sumithra Chandra.,Antimicrobial activity of *Terminalia catappa Manikara Zapota and Piper beetal leaf extract*, Indian J Pharma Science ,May-June:70(3):390-3, (2008).
6. Crag G M D J Newman- Annals of the New York Academy of Sciences 953(1):3-25, (2002).
7. Suryakanth chakradhari.,Parishruk Rajhans., Khageswar songs patel.,Erick K Towett.,Nutritional and spectral characteristics of Terminalia plant , ,EJMP(27), (2019),
8. Marimuthu Krishnaveni., G. Krishnakumari.,C.Ragina Banu.,M.Kalaivani., GC-MS Analysis of phytochemical in Terminalia catappa antimicrobial assay Indo American journal of pharmaceutical Research,(2015).
9. Pandey H., Chandra B., Nadeem M., Kumar A., Nandi S.K., Palni LMS In vitro propagation of some alpine medicinal herbs of the Himalayan region, Gyanodaya Prakashan, Nainital: 297–323,(2002).
10. Seema Pandey., Jaiswal V.S., (2002), Micropropogation of Terminalia arjuna Roxb from cotyledonary nodes, International Journal of Experimental Biology, 20:950-953.
11. Robert, M. L., Herrera, J. L., Chan, J. L., and Contreras, F., Micropropagation of Agave ssp. (Henequen), in Biotechnology in Agriculture and Forestry, 19 (Bajaj, Y. P. S., ed.), Springer-Verlag, Berlin Heidelberg, pp 305–329 (1992).
12. Went F W., On growth accelerating substances in the coleoptiles of arena sativa Proceedings of the section of sciences, Konin Klijee Academie van Wetenschappen re Amtesdam,30(10):10-19, (1926),
13. Haberlandt G., Physiological Plant anatomy Macmillan, London: 777,(1914).
14. Vishal P.,Deshmukh.,Prashanth V Thakare.,Uddhav S.,Chaudhari., Prashanth A.,Gawande and Vinod S.Undal., Assesment of Genetic Diversity among *Terminalia* species using RAPD Markers, Global Journal of Biotechnology and Biochemistry,vol 4 (2):70-74, (2009).
15. Opara FN., (2012) Preliminary Phytochemical screening and Antibacterial activities of leaf extracts of *Terminalia catappa,* Journal of Emerging trends in Engineering and Applied Sciences (JETEAS), 3(3):424-428.
16. M Weerawatanakorn,Supatcha Janporn,Chi Tang Ho and Visith Charavasit,T-*Terminalia catappa* linseeds as a new food source,J Science Technology,37(5),507-514,Sept-oct 2015.
17. M K Rai,N ,Shekhavath , Harish,A K Gupta,M Phulwaria K Ram,Plant cell,Tissue and Organ culture,106(2),179-190.