VIRTUAL WORKSHOP

CLONAL PROPAGATION OF TREE SPECIES

7th OCTOBER, 2020





ABSTRACTS

Institute of Wood Science and Technology
(Indian Council of Forestry Research and Education)
Bengaluru

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Bengaluru - 560003.

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(Indian Council of Forestry Research and Education)

Malleswaram, Bengaluru-560003

Preface

The Institute of Wood Science and Technology (IWST), Bengaluru is one of the research institutes of the Indian Council of Forestry Research and Education (ICFRE), Ministry of Environment, Forests and Climate Change, Government of India. IWST has a mandate to conduct research on wood science and technology as a national objective, and focuses its research activities to important forestry research needs of the states of Karnataka, Andhra Pradesh and Goa at regional level.

Clonal forestry is often seen as modern biotechnology. It is true that some techniques used for creating clones are new. The technical capacity to produce clones is improving. The current workshop aims to review the possible advantages of clones in forestry and to give information about the occurrence of clones in nature and forestry. Some specific attention is given to focus on matters, which are relevant for cloning as a tool to get quality wood. For any successful and healthy plantation, the two key factors are quality planting material and good management practices. In India, other than primary timber species, numerous other tree species are in demand for use as construction material/ for plywood industries. The dearth in quality planting material, both in terms of genetic strength and phenotypic vigor are the issues to be addressed. Both macro- and micro-propagation techniques can be efficiently employed in improving the production of quality planting material, and also the production of elite secondary metabolites of species which are under threat.

This abstract book includes all the abstracts received for the virtual workshop on CLONAL PROPAGATION OF TREE SPECIES, 7th October, 2020, from different institutes of India. They are segregated in two broad themes that include sub-themes covering both vegetative and *in vitro* propagation of tree species.

I hope this virtual workshop will help in exchange of ideas and dissemination of knowledge/skills in different clonal propagation options *viz.*, macro-propagation, *in vitro* propagation, conservation, *in vitro* secondary metabolite production and *in vitro* genetic modification.

I would like to place on record my appreciation for the excellent efforts put in by all the organising committee members.

(Dr. M. P. Singh, IFS)
Director, IWST.

CONTENTS

Theme 1

VEGETATIVE PROPAGATION OF TREE SPECIES

Sub-theme (i): Macropropagation techniques

EFFECT OF INDOLE-3-BUTYRIC ACID ON ROOTING OF *ACACIA AURICULIFORMIS* STEM CUTTINGS

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Acacia auriculiformis is a fast-growing, high biomass yielding and multipurpose tree species which grow up to a height of 30 m with a trunk diameter up to 60 cm in natural stands. The wood is principally used for construction, making furniture, firewood, paper and pulp. The species is also used for providing shade, windbreaks, and reduce soil erosion in different agroforestry systems. Rooting of stem cuttings is an important vegetative propagation method useful for large scale multiplication, establishing plantation with increased productivity and conservation of genetic resources. The present investigation was carried out to assess the effect of IBA on rooting and shooting potential of *A. auriculiformis*. The experiment was carried out in Completely Randomized Block Designs at Clonal Propagation Complex, GTI Division, IFGTB, Coimbatore. The shoot cuttings of 15-20 cm length and 5-6 mm diameter with buds were collected from the Vegetative Propagation Garden. Leaves were excised from the cutting and disinfected with Bavistin (2%) solution. The basal ends of the cuttings were treated with different concentrations of Indole-3-Butyric acid *viz*., 500, 1000, 2000, 3000, 4000 ppm for 30 seconds and planted in the root trainer filled with vermiculite as a rooting substrate, placed inside the low-cost polytunnel. The significant differences were recorded on percentage of callusing, number of sprouts, percentage of rooting, shoot length, root length, number of roots and percentage of survival. Among different concentrations the cuttings treated with IBA at 2000 ppm recorded higher callusing (85%), no. of sprouts (8), percentage of rooting (80%), shoot length (53cm), root length (36cm), no. of roots (primary root- 6, secondary root- 26) and percentage of survival (75%).

Key Words: Stem cuttings, rooting rate, IBA, callusing

EFFECT OF INDOLE-3-BUTYRIC ACID IN CLONAL PROPAGATION OF *AILANTHUS EXCELSA* ROXB.THROUGH COPPICE SHOOT CUTTINGS

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Ailanthus excelsa Roxb. is an indigenous, fast growing, multi-utility tree species belonging to the family of Simaroubaceae, commonly called as tree of heaven. Tree can grow between 18 to 25 m tall and girth of 3 m with cylindrical bole in suitable environmental conditions. Wood of *A. excelsa* is extensively used for safety matches industries for making matchwood boxes and match splints. Cottage industries are making wooden toys, cheap quality cricket bats by this tree. Wood is used as poles, pulp and paper, and also used for packing cases, fishing floats and sword sheaths. It is used as Grade III and Grade IV plywood. It is one of the most preferred species for safety match industry for wood used to good quality splints production. Safety match industry requirement is about 10,000 tonnes per month but the wood availability was only around 100 to 250 tonnes. There is a huge demand of *A. excelsa* wood in plywood and match wood industry. Farmers and match industries are embarked to grow *A. excelsa* in various methods of farming, due to its rapid growth, short rotation (utilizable girth attained in 5-7 years), marketing potential, low investment and substantial revenue. Good quality planting stock is a gap between demand and supply.

The rooting of cuttings will play important role for production of true to type and quality planting material from the selected phenotypes of *A. excelsa*. Vegetative multiplication plays important role on conservation and deploying large scale multiplication of elite genetic resources of tree species. Rooting of coppice shoots is cost effective and convenient method for mass multiplication for selected tree species. The study describes the scopes of clonal propagation in *A. excelsa* through coppice shoot cutting treated with IBA. The experiment was conducted to assess the rooting ability of *A. excelsa* with four replications; for each replication hundred cuttings were used. Lower parts of the coppice shoots were treated with different concentration (250, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 and 5000 ppm) of Indole -3- butyric acid (IBA) by powder dip method. Treated coppice shoots were immediately transferred to root trainer with vermiculite used as a rooting substrate. Treated coppice shoots were kept inside the low cost poly tunnels which maintains 90% relative humidity and temperature between 35^0C and 38^0C. The study exposed significant difference of rooted cuttings among the treatments. The highest (65%) rooting percent was observed in cutting with 2000ppm IBA treatment and the lowest (31%) with 500ppm IBA concentration. The use of 0.2% IBA treatment is suggested for rooting of juvenile coppice shoot cuttings of *A. excelsa*.

Keywords: *Ailanthus excelsa*, coppice, Indole-3-butyric acid, cuttings

SOFT STEM CUTTINGS WITH APICAL TIP FOR RAPID PROPAGATION OF *ANNONA MURICATA*

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Annona muricata L. is an important natural medicinal fruit tree among 70 species of Annonaceae family. *A. muricata* is dominantly cultivated all over the world, due to its huge demand and marketing price for its anti-cancerous property rich, leaves, fruits and bark. Out of nutritive aspects, currently studies focused elaborate specific to *A. muricata* and reaches in the level from common fever to anti-cancer treatment. Hence, vegetative propagation of biocontent rich trees has been suggested to produce beneficial products out of it. Comparing any other method, stem cutting propagation methods denote a simple, speed, low cost one. In Annona, rooting depends upon the physiological status of the cutting, and season is a major barrier in adventitious root formation. Cuttings were collected from various regions of Tamilnadu and Kerala. Stem cuttings were propagated in different mediums with IBA treatment in various concentrations. Apart from hard wood cuttings, soft wood cutting with apical tip shows better result when treated with IBA of 2000 ppm concentration. Environmental condition plays a vital role in the duration of emergence of shoot/root proliferation as well as success rate of clonal propagation in *A. muricata*.

Keywords: *Annona muricata*, apical tip, proliferation, clonal propagation.

EFFECT OF PLANT GROWTH HORMONES ON PROPAGATION OF *CINNAMOMUM TAMALA* THROUGH STEM CUTTINGS

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Cinnamomum tamala (Buch.–Ham.) Nees. & Eberm. commonly known as Tejpatta, Malabar leaf or Indian bay leaf is a promising medicinal plant species which is used in various Ayurvedic formulations, essential oils and for edible purposes due to its aromatic, astringent, stimulant and carminative properties. Owing to its economic importance and exhaustive harvesting practices, this species is being depleted at a fast rate. Production of quality nursery stock of this species through vegetative propagation will not only help in its conservation but also its commercial exploitation by local communities. Keeping this in view, the current study was conducted in Baragaon nursery, Shimla (H.P.) and the stem cuttings were treated with different concentrations of various rooting hormones *viz.* IBA, IAA, NAA using basal long soak (500 ppm, 1000 ppm & 1500 ppm) and basal quick dip method (2500 ppm, 5000 ppm, 7500 ppm and 10000 ppm) along with control where no treatment was given. It was found that the treatment of cuttings with 2500 ppm IBA using quick dip method resulted in maximum sprouting and rooting percentage of 62.5% and 56.7%, respectively, which were significantly higher than the control (35% sprouting & 25% rooting) and all the other treatments. The best treatment was followed by 5000 ppm IBA using quick dip method (50% sprouting & 48.3% rooting) and 500 ppm IBA (45% sprouting and 41.7% rooting) by basal long soak method, respectively.

Keywords: *Cinnamomum tamala*, growth hormones, propagation, stem cuttings.

METHODS OF VEGETATIVE MULTIPLICATION IN EUCALYPTUS FOR LARGE SCALE PRODUCTION

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Eucalyptus is one of the fastest growing forestry species worldwide, large scale vegetative propagation of Eucalyptus for production of quality planting stock has become important for forestry based industries. Cutting technique plays an important role in large scale production, conventional method of rooting through nodal or stem-cutting has certain limitations such as intra-clonal variation due to topophysis, poor root system and quick loss of rooting ability. Current study highlights the cloning techniques used for mass production of planting stock.

Initially, the cuttings were mainly produced following nodal or stem cutting technique, but various constraints based on rooting percentage and rooting behavior led to shift the large scale production to apical mini-cutting technique of cloning. Apical mini-cutting technique provides extra production period, higher performance, and better rooting ability as compared to the nodal or stem cutting, it resolved the problems associated with rooting percentage and behavior in poor rooter species of Eucalyptus and raised the rooting percentage from 65 to 95 along with better root quality, and also established a conducive environment for collection of plant propagules. Moreover, planting material produced by this technique shows very less intra-clonal variation in the main field as well as there is no requirement of rooting hormone and resulting into substantial cost reduction per cutting.

Further advancement in mini-cutting technique resulted into micro-cutting, where reduction in size of cutting made a huge economic difference and reduced the price of each cutting. Moreover, smaller cuttings are easy to transport and maintain during off-season.

Keywords: Cloning, nodal/stem cutting, mini-cutting, micro-cutting.

PHASES OF ADVENTITIOUS ROOT FORMATION IN *GMELINA ARBOREA* ROXB. SHOOT CUTTINGS

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The mechanism of adventitious rhizogenesis in shoot cuttings of woody perennials remains very poorly understood constituting an impasse in development of efficient cloning procedures for most trees of forestry origin. Thus, this study was undertaken to elucidate the process of adventitious root formation at physio-biochemical level taking *Gmelina arborea* Roxb., as a test tree. The levels of endogenous moisture content and biochemicals (auxin, soluble sugar and starch, phenols and o-phenol, peroxidase activity) were monitored at different stages of adventitious rhizogenesis in semi-hardwood (Day 0, Day 1, Day 3, Week 1, 2, 3, 4, 5 and 6) and miniature sprout cuttings (Day 0, Day 1, Day 3, Week 1, 2 and 3). Profound changes in endogenous biochemicals were registered during the adventitious root formation especially in the first 24 hours, indicating incidence of consequential process(es), which influence subsequent rhizogenesis in auxin-treated cuttings but not in non-treated ones due to inadequate endogenous auxin. The findings indicate towards two possible phases of adventitious rhizogenesis in shoot cuttings of *G. arborea*– the initial phase exhibiting low endogenous moisture and o-phenol content but elevated phenol and peroxidase activity and the later phase coinciding with higher endogenous moisture and low phenol content and peroxidase activity, separated by an intervening unstable duration which possibly varies among different type of cuttings.

Keywords: Auxin, endogenous biochemical, phenols, rooting, sprout cuttings.

PROPAGATION TECHNIQUE OF *LITSEA GLUTINOSA* (MAIDA LAKRI) - AN ENDANGERED SPECIES OF INDIA

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Litsea glutinosa (Lour.) C.B. Robinson is semi-evergreen, multipurpose, drought resistant, dioecious tree species which belongs to family *Lauraceae* and commonly known as Maida-lakri. Species is native to India, Southern China, Malaysia, Australia and the Western Pacific islands and found throughout Asia, including several regions of China, India, Bhutan, Myanmar, Nepal, the Philippines, Thailand and Vietnam. It grows at an altitude of 500-1900 m amsl. This species is under endangered category, red listing and critically endangered in some part of India. Bark is used for stomach disorder and to treat the diarrhorea and dysentery. Bark is also used in animal health care. Decoction of bark is applied to sores, scabies and to aches and pains. In pharmaceutical industry, bark mucilage gel is used as a binding agent for tablet formation. Population of this species is reducing in their natural zone. Owing to its high medicinal value and being an important ingredient of the pharmaceutical industry, the demand of this species is increasing day by day and the species is being exploited from its natural pockets illegally. Therefore, species has been recommended for *in-situ* as well as *ex-situ* conservation with devising appropriate management plan.

Seed germination of *Litsea glutinosa* was completed within six weeks with 30.04 per cent germination in nursery conditions. However, seeds treated with gibberellins (500 ppm) gave 41.48 per cent germination under controlled conditions. Two years old plants were fit for planting out in the field. Air - layering trials were carried out during March to May and developed the callus but rooting was not initiated. Shoot cuttings prepared from juvenile plants gave 43.00 per cent rooting. Macro - proliferation technique was developed for mass multiplication of this endangered species which is found cost - effective, eco - friendly and innovative.

Keywords: *Litsea glutinosa* (Maida-lakri), seed germination, macro-proliferation technique.

OPTIMIZATION OF VEGETATIVE PROPAGATION OF SANDALWOOD THROUGH ROOT SUCKERS

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Mass multiplication through vegetative propagation is an indispensable production tool to meet the requirements of wood based industry. In the last few decades, application of clonal technology has facilitated enhancement of productivity with improved wood quality. The success of clonal plantations was demonstrated in Eucalypts, Casuarina, Teak and other tree species. However, in spite of decades of research in Sandal, the mass multiplication either through cutting propagation or tissue culture has shown limited success due to unavailability of optimized and tested protocol for routine multiplication and field application. Vegetative propagation through stem cuttings, grafting, air layering and root suckers has been attempted. However, the rooting percent of stem cuttings was low at 15-20%. The present study was conducted to optimize propagation from root suckers collected from natural populations. The thickness of the root suckers, propagation in mist-less chamber, IBA concentration, selection of host and potting medium were optimized for germplasm collected from Koraput (Odisha) and Bargur (Tamil Nadu). This technology will facilitate establishment of clonal plantations in sandal.

Keywords: Sandalwood, vegetative propagation, root suckers

VEGETATIVE PROPAGATION OF TREES THROUGH ROOT AND LEAF CUTTINGS

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Vegetative propagation backed up with long term breeding programs and sound silvicultural practices is transforming clonal forestry in many countries around the world. The practice is finding acceptance for capturing the existing genetical potential of selected elite trees for replicating them in large number and their use in plantations for improving wood quality and productivity. Different methods of vegetative propagation viz., macro-propgation (stem cuttings, budding/grafting, layering, rhizomes etc.), micro-propagation (tissue culture, embryogenesis etc.) and combination of both have now been used in operational forestry and tree improvement based on tree species and conditions related therewith.

Of late, the interest is increasing in use of root and leaf cuttings in mass propagation of selected trees. The application of these propagules is, though species specific and have some inherent biological limitations in certain species, yet finding acceptance in some tree species especially those which produce suckers. Roots of some tree species on their severance from mother trees or wounding or exposure to sunlight transform some of their tissues into vegetative buds which start generating shoots. Full leaf with its stalk or portion of leaf with its veins when inserted in rooting media under favorable conditions reproduce roots those in due course also form shoots in certain tree species. The author has worked on leaf cuttings in *Dalbergia sissoo, Paulownia* species, *Populus* species including *P. gamblei* whose cuttings are difficult to root and root cuttings in *Populus* species, *Dalbergia sissoo*, *Paulownia*, *Melia dubia*, *Guava* etc. There are now reports that leaf cuttings are also used for mass propagation of *Syzizium cumuni* and some other species. The presentation gives details of reproduction using these propagules.

Keywords: Vegetative propagation, root cuttings, leaf cuttings

Theme 1

VEGETATIVE PROPAGATION OF TREE SPECIES

Sub-theme (ii): Clonal propagation of superior genotypes

SELECTION AND CLONING OF *ANOGEISSUS ACUMINATA*

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Anogeissus acuminata (Roxb. ex DC.) Wall. ex Guill. & Perr., commonly known as Phasi, enshrined in the socio-religious milieu of Odisha for its utilization in preparation of chariot wheels of Lord Jagannath Rathyatra of Puri. However, good trees of the species have been selectively logged for car timber in past centuries resulting in severe genetic degradation. Therefore the present study was undertaken for conservation and improvement of Phasi through selection of candidate plus trees (CPTs) and standardization of clonal procedures for multiplication of superior genotypes. No prior CPT selection was carried out in Odisha. Around 30 CPTs were selected in Nayagarh, Boudh and Satkoshiya Divisions employing comparison tree method with a selection intensity of 1 per 1000 trees, still the selected individual may not represent the true worth of the species. It would be fruitful to have early selections at the age of 12-15 years in the existing plantations, especially in promising plantations of Nayagarh division. No conventional methods of vegetative propagation are available for the species. Remarkable success has been achieved in clonal plantlet production from mature trees (as old as 60-70 years) through root cutting planting in mist chamber. The range of success was 35% to 72% in different candidate plus trees. The procedure is remarkable for its merit as clonal method. Shoot cuttings were collected for experimentation on adventitious root induction employing various treatments of auxins (IAA, IBA, NAA) and non-auxin growth regulators (thiamine, ascorbic acid, boric acid, pyridoxine). Juvenile seedling cuttings have shown 60-70% rooting. However, semi-hardwood cuttings from mature trees have low rooting ability (<20%). The best treatment for root induction was 5mM (around 100ppm) quick dip of Indole Butyric Acid (IBA). Thus, a Vegetative Multiplication Garden has been established with clonal stock of 9 CPTs at IFP Ranchi to optimize root induction through rejuvenation due to regular hedging of plants. Pilot experiment with 2400 cuttings revealed more than 55% rooting with 2mM (around 400 ppm) IBA quick dip treatment in mist conditions.

Keywords: Auxin, endogenous biochemical, phenols, rooting, sprout cuttings

PROPAGATION THROUGH JUVENILE CUTTINGS IN DIFFICULT-TO-ROOT *DALBERGIA LATIFOLIA* ROXB.

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Propagation of selected superior trees for quality planting material is one of the widely used practices for good adaptability and higher productivity in forestry. *Dalbergia latifolia* Roxb. (Family-Fabaceae) is a premium-quality high priced timber species internationally known as "Indian Rosewood". The species is classified as vulnerable (VU) in the IUCN Red list and categorized as "difficult to root" in literature with <10% rooting. Despite high market value, its occurrence is sporadic and plantations are 'rare' in central India. Its high density timber darkens with age and weighs about 850 kg per cubic meter. However, the species bears the constraint of poor propagation through shoot cuttings necessitating development of efficient clonal propagation procedure for large scale quality planting material.

Progeny of 10 selected superior trees of *Dalbergia latifolia* was raised in the Institute campus for propagation through juvenile shoot cuttings and hedged yearly in Ist/IInd week of April. Juvenile shoot cuttings i.e., extension growth of one year was used for the experiment. With varying rooting ability of progenies, an average of ≈35% adventitious rooting was achieved in shoot cuttings from 1-2 year old progenies with basal dip treatment of 2.0mM IAA which gradually decline in successive years. 4-5 years old progenies exhibited ≈30% adventitious rooting with basal dip treatment of 5.0mM IAA. Further reduction in adventitious rooting was recorded in cuttings from 8-9 years old progenies that exhibited ≈26% adventitious rooting with basal dip treatment of 5.0mM IAA + 1.0mM Boric Acid. Besides several endogenous and exogenous factors, transport and availability of exogenously applied rooting hormone to the target cells in pericyclic and endodermis region substantially affecting induction of adventitious rooting. Therefore, use of juvenile shoot cuttings from progenies of selected trees is an adoptable option for production of quality planting stock of *Dalbergia latifolia*.

Keywords: Adventitious rooting, IAA, progeny, shoot cuttings, *Dalbergia latifolia*.

STANDARDIZATION OF MACROPROPAGATION THROUGH ROOT CUTTINGS IN *DALBERGIA LATIFOLIA* (INDIAN ROSEWOOD)

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Dalbergia latifolia Roxb. is an economically important timber tree species of India. Because of over-exploitation and other ecological factors, the population of this tree has been dwindling. Hence, *D. latifoila* has been categorized as "Vulnerable" in the Red Data Book of IUCN. Therefore, there is urgent necessity to conserve the valuable genetic resources available in the country to prevent its deterioration as well as for initiating genetic improvement programs. In this context, standardization of vegetative propagation will go a long way to preserve the genetic resources and to produce quality planting stock. The superior trees identified from the natural populations of *D. latifolia* located in Palakkad, Nemmara, Chalakudy and Malayattoor Forest Divisions of Kerala as well as Coimbatore and Theni Forest Divisions of Tamilnadu were taken up for the study. The root cuttings from the superior trees were collected and buried in nursery beds filled with sand, watered and maintained under polytunnels, for producing sprouts. The sprouts of different lengths (5 – 10 cm, 11 – 15 cm and 16 – 20 cm) produced from the root cuttings were treated with IBA at 2000 ppm and 4000 ppm concentrations, by basal dry dip (powder form of IBA) and basal quick dip (liquid form of IBA) methods. The sprouts treated by basal dry dip method were placed in vermiculite and coir pith rooting media. The sprouts treated by basal quick dip method were placed in water, sand and red soil media. The different lengths of sprouts did not show any significant difference in the production of roots. The IBA 2000 ppm concentration resulted in high rooting of sprouts, compared to IBA 4000 ppm, in both basal dry dip and basal quick dip methods. The vermiculite and water media showed high rooting percentage in basal dry dip method and basal quick dip method respectively. The age of trees (based on GBH) did not show any significant difference in number of sprouts produced and rooting percentage. But, the age of trees showed significant difference in the survival of rooted plant-lets; the plant-lets developed from younger trees had high survival rates, compared to the ones developed from older trees. The studies have shown that selection of superior trees of optimum age, choosing of appropriate rooting medium and application of right concentration of IBA are some of the critical factors for ensuring high rooting of sprouts and survival of rooted plant-lets in *D. latifolia*.

Keywords: Indian Rosewood, macro-propagation, conservation, genetic resources, genetic improvement.

QUALITY PLANTING STOCK PRODUCTION OF *GMELINA ARBOREA* ROXB.

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Gmelina arborea (Gambhari) is an indigenous, fast-growing and multi-utility tree species belongs to the family of Lamiaceae. Wood is used for plywood, pulp, Particleboard, Veneer, Structural uses, Carpentry and Packing. It is also used for carving, board making, fuelwood and charcoal. The roots, fruits, leaves, bark, and flower is used for treating the scorpion sting, snake bites and diabetes. The success of large-scale plantation program fully depends on the planting of quality planting stock. Clonal propagation of selected genotypes will help to capture the full genetic potential of existing variation and increase the productivity of the plantations. The present experiment was conducted to investigate the effect of different concentrations of Indole-3-Butyric Acid for rooting and sprouting behavior of Gmelina cuttings. The rooting experiment was conducted in a Completely Randomized Block Design with four replications. In the present investigation effect of different concentrations of IBA was used along with control. Different concentrations of IBA viz., 100ppm, 250ppm, 500ppm, 750ppm, 1000ppm, 1500ppm, 2000ppm, 3000ppm, 4000ppm, and 5000ppm to assess the effect of IBA on rooting and survival potential of Gmelina. Cuttings were collected from the vegetative multiplication garden and treated with different concentrations of Indole-3-Butyric Acid. The cuttings were planted in the root trainer containing vermiculite and placed inside the low-cost polytunnel. Rooting and sprouting were recorded 35 days after planting. Among different concentrations, significant variations recorded on shooting, rooting and survival of cuttings. The cuttings were treated with IBA at 2000 ppm recorded higher rooting (75%), sprouting and survival percentage.

Key Words: *Gmelina arborea*, Indole-3-Butyric Acid, rooting, survival.

PRODUCTION OF QUALITY PLANTING MATERIAL OF *GMELINA ARBOREA* THROUGH VEGETATIVE PROPAGATION

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Gmelina arborea Roxb. (Family: Lamiaceae) commonly known as Gamhar is one of the species preferred by the farmers for plantations. It is valuable for furniture, paper and matchwood industry. Root of *Gmelina arborea* is an ingredient of the medicine Dasamula. It promotes digestive power and improves memory. With the aim of multiplication of phenotypically superior CPTs, semi hardwood cuttings of five different CPTs were collected from Barha Forest Division, Jabalpur. The cuttings of 10-15 cm length were prepared and quick dip treatment in talcum powder paste (10gm) was applied with different concentrations (control, 100 ppm, 200 ppm, 500 ppm and 1000 ppm) of IBA. The morphometric parameters of number of sprouts, length of sprouts (cm), rooting percentage, number of roots and root length was recorded. Recorded data was subjected to two factor statistical analysis. After 30 days, significant effect of trees (genotypes) and interaction between trees and treatments were observed for sprouting parameters. Maximum numbers of sprout (7.497) were obtained in tree number 2 on control. The obtained length of the nodal sprouts was as follows: control (2.747cm) > 200 ppm (2.43cm) > 100 ppm (2.401cm)> 500 ppm (2.216cm) > 1000 ppm (1.993cm). The result for rooting % was significant for the treatments and best rooting percentage was obtained in 200 ppm IBA (73.13%). This was followed by 100 ppm IBA (56.73%) > 500 ppm IBA (50%) > 1000 ppm IBA (21.33%) > control (13.33%). It was concluded that increased number of sprouts were obtained without any growth hormone but maximum rooting percentage with maximum root number (6.22) and root length (6.21 cm) was achieved in the cuttings treated with 200 ppm IBA. It was also observed that sprouting and rooting were independent of each other and even a cutting with small sprouts could have well developed root system. Quality planting material of *Gmelina arborea* was produced through this method.

Keywords: CPT, *Gmelina arborea*, Indole-3-Butyric Acid, rooting, semi-hard wood cuttings.

ASSESSMENT OF *NEOLAMARCKIA CADAMBA* CLONES COPPICING ABILITY AND ITS ROOTING PERCENTAGE

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Neolamarckia cadamba is a tropical evergreen tree belonging to the family Rubiaceae. It is native to India, China, Australia, Malaysia, Papua New Guinea, Philippines, Singapore and Vietnam and it is found in semi evergreen and moist deciduous forests. *Neolamarckia cadamba*, exhibit less seedling vigour due to smaller seed size, hence seedlings establishment is a problem. Due to the poor seed year combined with less viability period and seedling vigour, coppice shoot cuttings is the best method for propagation in *N. cadamba*. In any tree improvement programme, high genetic gain in a shortest possible period of time is achieved through development of clone. This will lead to creation of clonal plantations with superior traits as that of their parent material. Best performing clones will not be suitable for release even if the productivity of clones is higher when its coppicing ability and rooting percentage is less or minimum. Hence to identify the high productive clones with higher coppicing ability and rooting percentage an experiment was conducted in Institute of Forest Genetics and Tree Breeding (IFGTB) at Coimbatore during 2017 to 2018. The coppice shoots were collected from different clones at regular interval and treated with IBA at 2000 ppm and we found that clones viz., C-15, C-44, C-60, C-112, C-115, C-116, C-141 and C-144 are producing more coppice shoots and has higher rooting percentage when compared to all other clones. These clones are suitable to test at multi-location clonal trial for further short listing the high productive stable clones across locations.

Key words*:* *Neolamarckia cadamba*, coppicing ability, rooting percentage clones

EFFECT OF GRAFTING SEASON AND CLONE FOR DEPLOYING COMMERCIAL PROPAGATION OF TAMARIND (*TAMARINDUS INDICA)*

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Tamarind is one of the most important multipurpose tree grown in farm land, backyards, bund and avenues. The acidic pulp is digestive, astringent, laxative, refrigerant antiseptic and used as main ingredient for many south Indian food preparations. Grafting is an important tool for propagation and conservation of tree genetic resources without altering genetic makeup of the species. This investigation aimed to study the effects of grafting season on the success and survivability of different Tamarind clones. Cleft grafting experiments were laid out in Completely Randomized Block Design with four replications in GTI Propagation Complex, IFGTB, Coimbatore. The experiment comprises of 12 grafting time viz., 15th every month from January 2019 to December 2019 and seven clones of Tamarind viz. IFGTBTI -1. IFGTBTI -2, IFGTBTI -3, IFGTBTI -5, IFGTBTI -7 IFGTBTI -9, IFGTBTI -12 were used. Significant variations on the number of days for bud break, number of shoots, shoot length, number of leaves per grafts, and final survival percentage were observed on the season of grafting. The minimum number of days for bud breaking (9.25 days) was observed in the 15th April followed by 30th April (9.78 days) while the maximum days for bud breaking (17.47 days) was recorded in 15th October grafted plants. Grafting carried out on 15th April showed high performance in number of shoots (5.25), shoot length (6.20 cm), number of leaves per grafts (30.50), and final survival percentage (85 %) whereas lowest values (35%) were recorded on 15th October. Among different tamarind clones, the highest survival percentage of grafts was recorded in IFGTB -5 (85%) followed by IFGTB -2 (82%) whereas the lowest percentage was in IFGTB -9 (64.50%) during April 15. It is concluded that the best season for cleft grafting of Tamarind for deploying large scale multiplication is April to May.

Keywords: Cleft grafting, propagation, bud breaking, survival, success rate

Theme 1

VEGETATIVE PROPAGATION OF TREE SPECIES

Sub-theme (iii): Conservation of selected germplasm

EX-SITU CONSERVATION OF RARE, ENDANGERED AND THREATENED (RET) PLANTS OF NORTH INDIA

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The present study was conducted for development of *ex-situ* conservation of five rare and endangered plant species of north India viz. *Catamixis baccharoides, Ulmus wallichiana, Rauvolfia serpentina, Berberis aristata* and *Mahonia jaunsarensis.* Germplasm consisting of 2 accessions of *Catamixis baccharoides,* 9 accessions of *Ulmus wallichiana,* 8 accessions of *Rauvolfia serpentina,* 8 accessions of *Berberis aristata* and 5 accessions of *Mahonia jaunsarensis* was collected from diverse sources in northern India. Treatment of cuttings with 6000 ppm Indole-3-Butyric Acid (IBA) was found effective for propagating *Catamixis baccharoides* and *Ulmus wallichiana.* In case of *Rauvolfia serpentina,* 7000 ppm IBA generally evoked better rooting response in comparison with other IBA concentrations and resulted in greater plant percent (45.0 to 56.3 per cent). The survival percentage of wildings of *Rauvolfia serpentina* was much higher (92.5 to 96.3 per cent). Root induction did not occur in cuttings of *Berberis aristata*. Air layering was employed in this species yielding maximum plant percent of 18.8 with IBA treatment at 5000 ppm. IBA at 4000 to 5000 ppm concentration was found optimum to induce rooting in cuttings and produce plants of *Mahonia jaunsarensis.* Rooting and plant percent of different germplasm accessions within a species generally did not vary significantly which suggests that these propagation protocols can be reliably applied for multiplying germplasm of these species from wide sources. The propagation techniques developed in this study can be used for setting up germplasm banks representing wider diversity of these species.

Keywords: *Ex -situ*, RET species, Indole-3-Butyric Acid

COLLECTION, PROPAGATION AND CONSERVATION FOR ELABORATION OF ITS MEDICINAL PRODUCTS OF *FICUS GLOMERATA*

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Ficus glomerata (Moraceae) is a familiar medicinal tree in India, which has long been used in Ayurveda, the ancient system of Indian medicine, for various diseases/disorders. Altogether, more than 1,200 species of birds and mammals eat figs around the world, and so they are a massively important food resource, and those animals are the dispersers of many other tree species. It is widely used to treat liver disorders, diarrhea, hemorrhoids, diabetes, asthma, hyperglycemia, diarrhea, to cure cancer etc. Our survey in villages, fringes of hill and wild: this species is managed *in situ* and not cultivated. The edible fruits highly appreciated by the indigenous people of villages. Due to biotic and abiotic factors and encroachers this tree is going to become vulnerable soon. In view of conservation and characterization of this important germplasm for its active molecules, survey has been done and from the collected germplasm vegetative propagation was done through stem cuttings. Various growing medium were tried and vermiculite medium was found to be a suitable one. The percentage success of rooting was 20-50%. Rooting percentage varied with thickness of cuttings. Higher rooting was noticed in 10-20mm sized cuttings. The stem cuttings were treated with Indole-3-Butyric Acid, 2500 ppm - 3000ppm showed higher root induction than other concentrations. Within 45 days it was able to observe the root growth and shoot growth. This research showed better growth of stem cuttings of *Ficus glomerata*. We hope this macro-propagation methods are not only an alternative means for mass proliferation of this valuable tree germplasm but also help in the conservation for future use and saving biodiversity.

Keywords: Propagation, root trainers, rooting, conservation, *Ficus glomerata*.

SOFTWOOD GRAFTING FOR CONSERVATION OF TAMARIND

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Throughout tropics, tamarind (*Tamarindus indica* L.; 2n=24=2x) is an economically important species. It is grown unattended in backyards, roadsides or wastelands. It is a multipurpose tree for agroforestry system. However, the economic benefit from tamarind to dependent communities is threatened as the species' natural populations face depletion due to over-exploitation, habitat loss, and absence of systematic conservation strategies. Thus, conservation of tamarind germplasm *in-situ* and *ex-situ* is critical regionally for livelihoods of the people dependent on it and also globally for the preservation of important genetic resources. Keeping this in view, survey and exploration for tamarind from tribal areas of Telangana, Chattisgarh, Odisha and Mizoram were made to identify superior genotypes with attractive traits such as long pod length, sweet and sour pulp and high pulp recovery. The scion wood of *in-situ* identified superior trees was collected and brought back to IIHR, Bengaluru to be multiplied and established in the germplasm block of tamarind at the institute using softwood grafting technique. The rootstocks were raised from tamarind seeds collected from local trees during the harvest season of April-May. The seeds were extracted from ripe pods, washed with water and dried to be used at a later date. Pre-treatment of seed by soaking in water for 24 hrs prior to sowing was found to improve germination by 80 percent. The seeds are sown in nursery bags and seedlings were ready for grafting in 9 months. The scion wood having approximately same width as the rootstock was selected from a terminal shoot of the superior trees from the surveyed area. It was kept moist throughout by storing in wet gunny bag till the time grafting was performed, sometimes as many as 4 days. The grafted plants were kept in polyhouse with intermittent mist spray. This method gave success rate of 25 percent and the time of collection of scion wood was found to be the most important factor contributing to the success. There is a need to develop better methods of storing the collected scion wood so that it retains its viability for longer time making the collection and conservation efforts more fruitful.

Keywords: *Tamarindus indica*, softwood, grafting, conservation.

Theme 2

IN VITRO PROPAGATION OF TREE SPECIES

Sub-theme (i): Micropropagation techniques

IN VITRO SHOOT INDUCTION IN *DIOSPYROS BUXIFOLIA* (BLUME) HIERN

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Diospyros, one of the largest angiosperm genera, has more than 40 species in India with varied uses. *D. buxifolia* is a large ornamental, lofty, buttressed species of this genus, with 30m height and 1m diameter. In India, it is widely distributed in evergreen and semi evergreen forest of Western Ghats. Its wood is reddish grey, streaky, hard and close grained, and valued as a type of ebony. It is used in making excellent walking stick, poles, match boxes and splints. This species is conventionally propagated through seeds, which are produced once a year and have very short viability. Hence, there is a need for developing alternate propagation techniques for this species, especially using *in vitro* approaches, as macropropagation is less successful in *Diospyros* species.

D. buxifolia is the only timber species of the genus in which micropropagation is reported, wherein leaf and nodal explants were used for *in vitro* culture establishment. We studied the effect of concentrations and combinations of different plant growth regulators for *in vitro* shoot induction in *D. buxifolia*, and the effect of additives like PVP and activated charcoal in reducing explant browning and leachates in the medium. The bud break was observed in all media combinations (except in medium containing Zeatin and Ac. charcoal) within 22-35 days after the date of inoculation. Some nodal explants started drying (after 59 days) from tip (in medium containing Zeatin and PVP), while some turned black probably due to phenol exudation, but there was no leaching into the medium. After third subculture, some explants showed callusing at the base, while phenol exudation on the surface of the explants remained. It is expected that the development of micropropagation protocols will aid in the conservation and mass multiplication of this lesser-known slow-growing timber species.

Keywords: *Diospyros buxifolia*, *in vitro* shoot induction, plant growth regulators

FACTORS AFFECTING SUCCESSFUL *IN VITRO* CULTURE ESTABLISHMENT AND PLANT REGENERATION IN *MADHUCA LONGIFOLIA VAR. LATIFOLIA*

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Madhuca longifolia var. *latifolia* (Family Sapotaceae) is commonly known as Indian Butter tree or Mahua, is predominantly distributed in central, southern and northern regions of India. More than 70 % of the tribal population is engaged in collection, drying and selling of mahua flowers. It is commonly propagated through seeds but seeds are recalcitrant and susceptible to fungal attack. In the present study, a successful attempt was made to propagate Mahua through micro-propagation. Nodal segments were used as explants and they were collected every month throughout the year. Axillary bud break (64.44%) was successfully achieved by culturing the nodal segments on Murashige and Skoogs (MS) medium supplemented with 3 mg l^{-1} Benzyladenine (BA) collected during the months of July-September (rainy season). Different cytokinins were tried for shoot multiplication and maximum (2.8) number of shoots with 4.35 numbers of leaves and 4.78 cm shoot length was achieved on MS medium supplemented with 3 mg l^{-1} BA when a subculture cycle of 30 days was followed. Successful *in vitro* rooting (55.55%) was achieved within 40 days on medium supplemented with 2 mg l^{-1} Indole-3-Butyric Acid (IBA). The rooted plantlets were successfully hardened by using a two-step method on ½ strength of MS liquid medium in culture bottles and soilrite in root trainers. The hardened plantlets were acclimatized first in a mist chamber and then in polybags in shade house. In the present study *in vitro* shoot regeneration and plantlet formation through nodal segments of *Madhuca longifolia* was achieved.

Keywords: Axillary bud, benzyladenine, Indole-3-Butyric Acid, *In-vitro*, *Madhuca longifolia.*

MICROPROPAGATION IN MORINGA FOR HIGH-THROUGHPUT MULTIPLICATION

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Moringa oleifera is the most widely cultivated species of the Moringaceae family attributed with several medicinal uses and a high nutritional value. This plant is native of the western sub-Himalayan and now distributed worldwide. The current investigations have been attempted to establish the *in vitro* micro-propagation protocol of *Moringa oleifera* (Variety-PKM-1) from nodal sections of young *ex vitro* grown seedlings. Nodes were cultured in Murashige and Skoog (MS) medium with various combinations of plant growth regulators. Multiple shoots were successfully achieved by culturing nodal explants in medium containing different concentration of 6-Benzylaminopurin (BAP) and Kinetin (Kin) in combination with naphthalene acetic acid (NAA). BAP at 2mg/l was considered optimal for generating more axillary shoots per explants after 30 days of inoculation. A high rate of multiplication has been established by routine subculture on a similar shoot induction medium. *In vitro* rooting on a medium containing indole-3-butyric acid (IBA) at 1.5mg/l was maximum for individual shoot culture. After being transplanted into the soil, seventy per-cent of the rooted plants survived, provided that the potted plantlets were covered with clear polythene bags and kept 15 days before exposure to ambient conditions in a shaded greenhouse.

Keywords: Micropropagation, moringa, benzylaminopurin, naphthalene acetic acid, kinetin.

Theme 2

IN VITRO PROPAGATION OF TREE SPECIES

Sub-theme (ii): Production of quality planting material

IN VITRO CLONING OF *TECTONA GRANDIS* L. IN DIFFERENT GENOTYPES OF KARNATAKA FOR HIGH QUALITY PLANTING MATERIAL

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Tectona grandis L. (Teak) is one of the most valuable timber species of the world. India is considered to be the centre of diversity for teak and it is widely distributed in Central and South India. The increasing demand of teak for plantation purposes by forest department as well as private companies has necessitated research on unconventional methods for the improvement of productivity. Clonal propagation of superior genotypes through *in vitro* method is essential for rapid genetic improvement. Therefore *in vitro* propagation method plays an important role for commercially feasible of teak by producing genetically uniform plants from selected genotypes. This paper describes a method for *in vitro* cloning of phenotypically superior individuals of teak using nodal shoot segments as explants. Five different genotypes (HAL-42, TMT-21 & 22, CHI-47 and SHI-08) were selected from four different regions of Karnataka (Haliyal, Titimati, Chikamangalur and Shimoga). Multiple shoots of high quality were produced from nodal explants of genotype collected from the region of Titimati area. Among the five clones tested, TMT-21 exhibited highest rate of shoot induction (>82%). Averages of 3-4 shoots per explants were obtained within 4 weeks of culture on MS medium supplemented with BAP 1mg/l. Rate of shoot multiplication also significantly differed in different genotypes. About 6 - 7 fold multiplication rate was observed in TMT-21 within 4 weeks period, when cultured on modified MS medium supplemented with BAP 1mg/l and Kn 0.5mg/l along with NAA 0.1mg/l. *In vitro* raised shoots could be successfully rooted (92.3%) on one fourth MS medium along with IBA 2.0 mg/l. *Ex vitro* rooting percentage was 85.67% when *in vitro* shoots were pulse treated with IBA 1500 ppm for 30 minutes. The plantlets were acclimatized in root trainers first in a mist chamber and then in polybags in a mixture of sand: soil: compost under greenhouse conditions. This new protocol will meet the high demand for quality planting material of teak for large scale plantations.

Key words: *In-vitro*, cloning, *Tectona grandis*, micropropagation, genotypes.

Theme 2

IN VITRO PROPAGATION OF TREE SPECIES

Sub-theme (iii): Clonal propagation for secondary metabolite production

PRODUCTION OF CAMPTOTHECIN FROM *NOTHAPODYTES NIMMONIANA* GRAHAM IN CELL CULTURES

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Nothapodytes nimmoniana Graham is an endangered, medium sized, medicinal tree belonging to the family Icacinaceae. It is distributed in the evergreen forests in Western Ghats of India, North-East India, Sri Lanka, Myanmar and Thailand. The tree growth is slow, and propagation is usually done by seeds. *N. nimmoniana* is a rich source of camptothecin (CPT) (isoquinoline alkaloid) which is an anti-cancer drug that holds a high commercial value. Naturally, camptothecin has been reported at higher yield from *N. nimmoniana* with greater quantity in stem and root of the tree, which led to the over harvesting and exploitation of this tree species. Hence, studies were under taken to establish the cell cultures (batch cultures) of *N. nimmoniana* for high yield of camptothecin (the elite secondary metabolite). Seeds of *N. nimmoniana* were sterilized and inoculated on hormone free medium for germination. The germinated seeds of different parts such as cotyledon, hypo cotyledon, leaf, and roots were used for callus initiation. Different combinations of hormones such as 2,4-D 1mg/l, 2,4-D 2mg/l, picloram 2mg/l, TDZ 0.5mg /l and 2,4-D 1mg/l+BAP2.5mg/l were used to initiate the callus culture. Leaf and hypocotyl explants showed better response compared to cotyledon and root. Established callus culture was multiplied and maintained up to 4-5 subcultures. Among all the combination of hormones tested, NAA 10.74µM+BAP 2.22µM was considered to have best cell culturing and 2,4-D 2mg/ml, 2,4-D 1mg /ml was good for cell growth. About 0.4g of cells (fresh cell weight) was transferred into a 100 ml Erlenmeyer flask with 20ml of MS medium supplemented with NAA 10.74µM+BAP 2.22µM. The cultures were shaken at 110 rpm and maintained at 25ºC with 8hrs photoperiod with constant shaking. To elute camptothecin into the suspension cultures elicitors like Chitosan was added to 18-day old cell cultures. The yield of CPT from suspension culture extracts was 90% pure when eluted and compared with Standard CPT by TLC.

Keywords: *N. nimmoniana*, camptothecin, secondary metabolite, cell culture, chitosan.

Theme 2

IN VITRO PROPAGATION OF TREE SPECIES

Sub-theme (iv): *In vitro* genetic modification

COMPOSITE TRANSGENICS AS A TOOL FOR FUNCTIONAL ANALYSIS OF GENES IN TREES

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One of the applications of tissue culture techniques is in generating transgenic plants. Transgenic tools are used for gaining insights on the functional role of genes in determining a given trait in the plant's genetic and physiological environment. In woody species, the low transformation efficiency and the time required for generating transgenic trees does not permit rapid functional evaluation of genes. Organogenesis and somatic embryogenesis routes used for regeneration of homogenetically-transformed plantlets from single transformed cells takes around 2 years. Therefore, approaches not involving these techniques have been developed. Composite transgenic approach involves generation of GFP tagged transgenic roots on non-transgenic shoots by infecting *Agrobacterium rhizogenes* under *in vitro* conditions. This approach has been used in conjunction with RNA*i* and CRISPR/Cas9 for rapid functional analysis of genes involved in root growth and development in difficult to transform plants like Casuarina (Gherbi *et al.,* 2008; Svistoonoff *et al.,* 2013). In Eucalyptus, the composite transgenic strategy was developed at IFGTB (Balasubramanian *et al.,* 2011). The high transformation efficiency (~ 60 %) and a less time consuming procedure of around 5 months required to generate hardened composite transgenic enables rapid analysis of the phenotypic effects of the transgene expression in roots. The tool has been successfully applied by Placensia *et al.* (2015) and Dai *et al.* (2020) for validating the function of the lignin biosynthesis gene *EgCCR1* in *E. grandis* using the techniques of RNA*i* and CRISPR/Cas9 respectively. We have also demonstrated the use of this technique for RNA*i* mediated functional validation of the *EcHKT1;1*gene for conferring salt tolerance. Composite transgenic strategy thus has potential application for rapid identification of genes to be chosen for genetic modification or in marker assisted breeding programmes for desired traits like drought, flooding and other less studied traits in trees.

Keywords: *E. grandis*, *Agrobacterium rhizogenes*, *in-vitro*, organogenesis, somatic embryogenesis

DEVELOPMENT OF POLYPLOIDY IN *SANTALUM ALBUM* L. USING *IN-VITRO* TECHNIQUE

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Sandal (*Santalum album* L.,) is one of the most important economic tree species; oil extracted from the tree is used preparations of perfumes, formulations, flavors, cosmetics, toiletries, beauty aids and medicines. Plant breeding techniques such as selection and hybridization has not been reported in this species for creating genetic variations. Polyploidy is one of the techniques for creating genetic variation which can be employed for improvement in desired traits. Artificially induced polyploids obtained during *in-vitro* micro-propagation are known to enhance production and/or qualitative improvement. Keeping this in view, an attempt is made to develop polyploidy in *Santalum album* through *in-vitro* techniques.

Two sets of experiments were conducted for duration of 24 h. One with lower concentration (15, 30, 45, 60, 75, 90, 105, 120, 135, 150 µm) and another with higher concentration (200, 250, 300 and 350 µm) of oryzalin were tested on *S. album* seeds. A oryzalin-free MS medium was used as control. Under aseptic conditions oryzalin was filter sterilized using 0.22 micron filters and 10 ml syringes. The filter sterilized oryzalin was added to MS liquid medium. The ploidy level was initially determined by morphological variation, namely, slower/faster growth, darker larger/small leaves, and healthy shoots in polyploid, compared with control seedlings.

Lower concentrations and shorter durations of exposure to oryzalin tended to have greater survival. Best germination (70 %) was observed when seeds were treated at the lowest concentration (45 µM) for the 48 hrs. Other treatments (15, 30, 60 and 90 µM) at the same duration (24 hrs) resulted in moderate germination rate but when the concentration was increased, explants germination rate declined sharply. Seeds treated with 105 and 120 µM concentration of oryzalin showed slight callusing. No germination was observed in any of oryzalin-treated seeds without seed coat. Most of the survived treatments produced healthy shoots *in-vitro*, but there was very little adventitious shoot production. Polyploidy plantlets were identified first by their morphological characteristics. Polyploidy plants presented different morphologic features, such as a deeper green leaf color, thicker and smaller leaves and fast growth rate compared to control. Also, Vegetative characteristics such as the length and width of the leaves were significantly different when compared with polyploidy plantlets. Explants producing multiple shoots were showing faster growth compared to control. It is uncertain if this fast multiplication was the result of the treatment regime or simply a suboptimal multiplication procedure; hence further studies need to be done and ploidy induction conformation has to be carried out.

Keywords: Polyploidy, *Santalum album*, *in-vitro* technique.