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Plant tissue culture: A technique for propagation and conservation of *Butea monosperma* (Lam.) Taub. var. *lutea* (Witt.)

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ABSTRACT

Plants with medicinal properties are a vital global source of medications that can save lives. The most important instrument for choosing, propagating, and preserving the rare and important species of medicinal plants is biotechnology. Because it contains multiple types of secondary metabolites, *Butea monosperma* has vast therapeutic capabilities that have earned it a prominent place in the pharmaceutical industry. The minimal seed viability, low germination of the seeds rate, and genetic heterogeneity of *B. monosperma* impede its propagation. The main obstacles to the long-term cultivation of this significant plant are overexploitation, significant habitat damage, and limited ranges. *Butea monosperma* is a prominent medicinal plant, and it's in vitro tissue cultivation and micro-propagation are well-established processes. For this particular plant species, the fast and repeatable in vitro response to treatments with plant growth regulators has become a crucial component of genetic transformation research. This chapter covers advances and improvements in the genetic transformation of *B. monosperma* as well as methods for in vitro regeneration. In conclusion, we offer recommendations and future directions for this significant species of tree with medicinal value.

Keywords: *Butea monosperma,* Micropropagation, Genetic transformation, Conservation

INTRODUCTION

Although it is a medium in size woody tree that grows throughout India, Bangladesh, Nepal, Sri Lanka, Myanmar, Thailand, Cambodia, Vietnam, Malaysia, and Western Indonesia, *B. monosperma* (Fabaceae) is commonly referred to as flame of woodland and bastard teak (Kirtikar & Basu, 1935). It is also highly significant medicinally (Firdaus & Mazumder, 2012). This tree grows to a medium height of 12 to 15 metres and is upright. Its entire plant has commercial and medical worth. Having been positioned for a specific purpose, this tree is the most beautiful and distinctive one. *Butea monosperma* has become a gem of contemporary medicine and is widely employed in Unani healing, Ayurveda, and homoeopathic treatment. It has been traditionally claimed to have qualities that are stringent in nature, resentful, alterative, sexual stimulant, a repellent, antiseptic, and antiasthmatic. Butea chew is a crimson discharge that is extracted from the bark. It has antifungal and antidiarrheal qualities, and it contains a lot of gallic and tannic acids (Gunakkunru *et al*., 2005). Many plant sections have been shown to have phytochemical substances with antimicrobial activities, including alkaloids, cyanogenic glycosides, phenolic compounds, flavonoids, terpenoids, tannins, and saponins (Thirupathaiah, 2007). *B. monosperma* seeds are additionally utilised for treating a variety of conditions, such as tumours, haemorrhaging piles, kidney stones, intestinal worms, abdominal problems, and inflammation (Anonymous, 1988). Additionally, extracts, parts, and separated elements from the seeds have been identified as having antiviral (Yadava & Tiwari, 2005), anthelmintic (Prashanth *et al*. 2001), and antibiotic properties (Mehta *et al*. 1983). Additionally, this tree's blooms are an excellent supplier of flavonoids and are known to be demonstrated to possess anticonvulsant (Kasture *et al*., 2000) and antihepatotoxic (Wagner *et al*., 1986) qualities. Additional uses for this tree species include dye, resin, wood, and fodder (Reddy *et al*. 2001). The Indian Coastal plateau represents the native ecosystem of *B. monosperma*. There are just about 100 plants in total throughout the plateau, indicating a relatively small population. *B. monosperma* is an uncommon and threatened therapeutic plant, according to the Biodiversity Evaluation Control Management Workshop for Therapeutic Plant of Andhra Pradesh the state of India. It is currently endangered as a result of the damaging collection of plant parts for firewood and medicinal purposes, the destruction of its natural habitat, and ignorance of its limited availability (Aileni *et al*. 2014). Additionally, this plant is propagated by seedlings (Tandon *et al*., 2003), however its viability and germination rate are low. Numerous researchers are using tissue culture techniques to cultivate this key plant for pharmaceuticals, *B. monosperma*, as a result of the plant's declining availability and rising demand worldwide. Therefore, it may be beneficial to preserve

germplasm and cultivate exceptional *B. monosperma* plants through in vitro clonal development for massive production.

New techniques the use of records-pushed optimization of subculture medium composition also display very applicable outcomes (Zhou *et al*., 2023), but it's miles proven in this statement that the absorbed utilize of modern proficiency on conventional plant physiology can consequence in relatively speedy elucidation and marked development of way of life strategies. Besides, a few of the accessible results and annotations also are based leading the matter-of-fact enjoy of the authors, got at some point of extra than 30 years of research in this discipline. The predominant objective of this evaluation is to expose that plant biotechnologists and related industries can use this manuscript to find a way to avoid pointless paintings on signs and symptoms by way of focusing at the fundamental regulatory mechanisms of plant development. In the existing evaluation, we are devoted to descriptions of some of these steps one after the other with a few realistic suggestions. By being sessile, plant life has evolved with a complicated network of structures and alerts to alter and coordinate their improvement. One of these strategies is the incidence of stem mobile niches which might grow to be a brand new organ or maybe a brand new plant, upon sure situations. in this day and age, plant shoot (less) and root meristems are by a conduct the greatest premeditated stem mobile niches, but plant tissue culture has chiefly changed from this commentary and the incidence of this phenomenon (Fambrini *et al*., 2023 & Wang *et al*., 2020). In roots, the controller mobile match up to the quiescent center, and WUSCHEL and its HOMEOBOX (WUS–WOX) appearance is a indication for this cellular sort (Jha *et al*., 2020 & Wan *et al*., 2023). Evidence has established the inter-connection among auxin and WOX5 pathways, and existing revise have also verified that WOX5 tempt TAA1-mediated auxin bio-synthesis (Savina *et al*., 2020).Organizer cells be capable of as well be fashioned from the ready cells in plant organs, which do now not unite with the xylem/vasculature and can't canalize auxin in this mode. In the case of xylem formation, handiest the root can shape. Screening that this position of genes is essential for plant renewal, ectopic WUS expression may, consequently, be a pertinent loom for superior plant revival (Lee *et al*., 2022).

Traditionally, seeds are used to reproduce *Butea monosperma* (Tandon *et al*., 2003). Nevertheless, low germination frequency and poor seed viability limit its spread. Aileni *et al*. (2014) have said that the plant species was also in danger due to the damaging harvesting of plant parts for firewood and medicinal purposes, and also because of the quick destruction of their surrounding areas. Therefore, it is imperative that efforts be made to conserve this kind of tree. Plant tissue culture has shown to be a potential strategy for the conservation and massive amounts multiplication of multiple species of forests trees (Erst *et al*., 2015 & Tippani *et al*., 2013). According to Sharma *et al*. (2014), plantlets that have been regenerated in vitro are susceptible to genotypic change at any point during their development. Therefore, it is crucial to use molecular markers to ensure the genomic authenticity of the artificially rejuvenated plantlets. For many tree species, the chromosomal stability of artificially rejuvenated seedlings can be effectively assessed using RAPD markers (Rani *et al*., 1995; Bharti & Vijaya, 2013; Osman *et al*., 2014). The goal of the present investigation was to establish a quick and repeatable artificial regeneration system employing cotyledonary node explants obtained from axenic sprouts of *B. monosperma* using various hormones, taking into account the paucity of literature on the subject. RAPD markers were implemented to assess the genetic durability of the in vitro plants. The technique used for tissue culture may act as a viable substitute for the objective of multiplying and conserving this important species of tree.

Due to the damaging removal of plant parts for firewood, medicinal purposes, and other purposes, along with the destruction of its natural environment and ignorance of its uniqueness, the albino variety is currently in danger of going endangered in nature. Furthermore, reduced rates of germination and viability make seed propagation of this plant difficult. To highlight their rarity in nature, nearly every seed planted in the experimental garden was found to be viable (Reddy *et al*., 2008). Because it has a larger genetic foundation than vegetative material, seed is a better source of plant material for conservation purposes. In vitro treatments can significantly increase germination when traditional methods result in low or no germination (Fay, 1992). In vitro studies of plants depend heavily on the media composition as well as the qualitative and quantitative characteristics of plant growth regulators. Consequently, in vitro plant tissue culture research requires the optimisation of these circumstances. In order to conserve and restore germplasm, a huge quantity of *Butea monosperma* var. Lutea can be quickly produced by developing techniques for in vitro seed growth and the growth of seedlings (**Fig. 1**). We were motivated to create a straightforward, repeatable, and improved in vitro seed production and seedling growth approach for globally threatened and valuable woody plant species because there are no papers currently accessible addressing seed germination protocols for *Butea monosperma* var. Lutea.

The current reproducible and repeatable approach was created in response to these issues and the enormous curiosity about plant tissue culture techniques. Its goal is to generate large-scale, desirable, true-to-type plants within a short amount of time.

Table 1. Summary of micropropagation studies on *Butea species.*

Title	Explant	Taxa	Reference
Jasmonic Acid: Enhancing SPF Potential in *Butea monosperma* Floral Variants Callus Cultures	Fresh and young leaves	*B. monosperma* (Lam.) Taub (BM)	Manali and Indu, 2023
In vitro seed germination and development of *Butea monosperma* (Lam.) Taub. Var. *lutea* (Willt.) : a step for rehabilitation	Mature seeds	*B. monosperma* (Lam.) Taub. Var. *lutea* (Willt.)	Aileni *et al*., 2014
In Vitro Approaches for Conservation and Sustainable Utilization of *Butea monosperma* (Lam.) Taub. Var. Lutea (Witt.) Maheshwari: A Highly Valuable Medicinal Plant	Mature sseds	*B. monosperma* (Lam.) Taub. Var. *lutea* (Willt.)	Yarra *et al*., 2018
Rapid *in vitro* Plant Regeneration From Nodal Explants And Assessment of Genetic Fidelity Using Inter Simple Sequence Repeats Markers in *Butea monosperma* (Lam.) Taub. var. Lutea (Witt.)	Nodal explant	*B. monosperma* (Lam.) Taub. Var. *lutea* (Willt.)	Rathnaprabha *et al*., 2017
In vitro conservation and genetic homogeneity assessment of *Butea monosperma* (Lam.) Taub. Var. lutea (Witt.) Maheshwari—A potential pharmaceutical legume tree	Mature seeds	*B. monosperma* (Lam.) Taub. Var. *lutea* (Willt.)	Yarra *et al*., 2016
Micropropagation of white palash tree (*Butea monosperma* [Lam.] Taub. Var. *lutea* [Witt.])	Dried pods	*B. monosperma* (Lam.) Taub. Var. *lutea* (Willt.)	Rathnaprabha *et al*., 2017

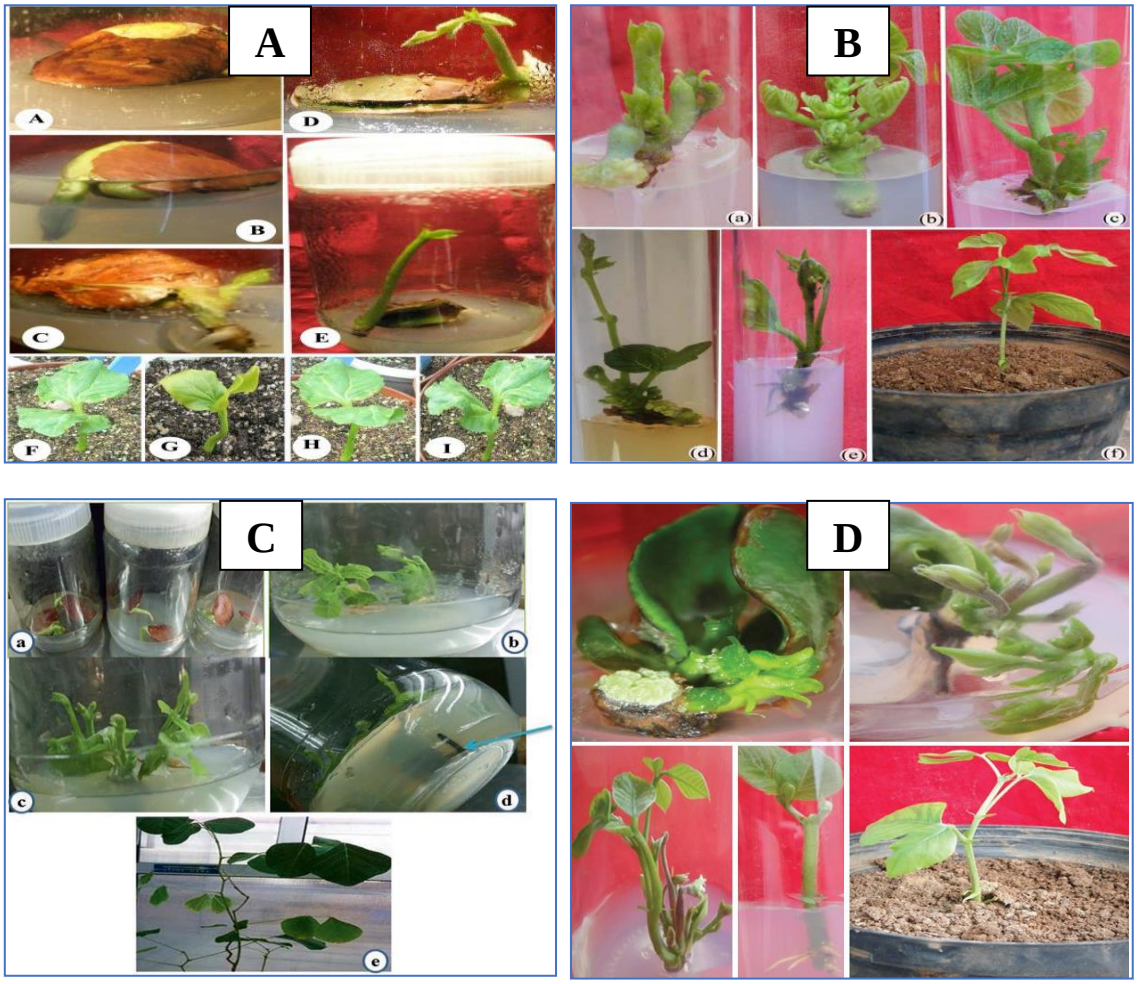

Fig. 1. [A] In vitro germination of *B. monosperma* seeds and seedling development A) After three days, cultured seed explants on MSF mixture treated with 4.40µM BA B), C), D) Various phases of seedling growth on MSF medium supplemented with 4.40µM BA After three weeks of incubation on MSF medium enriched with 4.40µM BA, the developed seedling is shown in E. Plantlets on soil in a greenhouse are shown in F, G, H, and I. **[B]** *B. monosperma* plantlet establishment and in vitro renewal from segments of nodal cortex In four weeks of

growth on MS enhanced with 2 mg L-1 BAP and 0.2 mg L-1 Indole acitic acid, (a) buds breaking from the nodal area of explants, (b,c) multiple shoot induction and proliferation from nodal explants of *B. monosperma*, (d) After a couple of weeks of colony on MS and 1.0 mg L-1 GA3, the shoots from the primary culture of *B. monosperma* elongated. (e) A *B. monosperma* rooted branch fed with 1 mg L-1 IBA; (f) A soil-acclimated plant. **[C]** *B. monosperma* micropropagation: (a) In vitro growth of seeds using MS basal medium at half strength. (b) Multiple shoots grown from cotyledonary node transplants obtained in vitro on MS media enriched with 4.44 µM BAP. (c) Shoot elongation with 4.44 µM BAP-augmented half-strength MS media. (d) Culture of shoots in vitro on ½ MS medium enriched with 2.46 µM IBA. (e) A greenhouse plant that has adjusted well. **[D]** Multiple branch expansion from cotyledonary nodal stimulates in vitro regrowth of *B. monosperma* (a) Multiple shoots are induced from cotyledonary node explants; (b) after 3–4 weeks of culture, multiple shoots proliferate on MS medium supplemented with 2 mg/L benzylaminopurine; (c) shoots elongate on the same proliferation medium; (d) shoots grown in MS medium augmented with 1 mg/L indole-3-butyric acid root; and (e) the entire plantlet is acclimatised in soil.

2. MICROPROPAGATION OF BUTEA

2.1. Culture Initiation:

Explant selection and isolation, surface sterilisation, and establishment on a suitable culture medium are typically included in the commencement of a culture(Rathnaprabha *et al*., 2017).

2.1.1. Explant Selection:

An explant is a section of plant tissue that has been extracted out and propagated. Reliance on some model systems, mainly tobacco, has led to the widespread assertion that any section of the plant can be used to cultivate a totipotent explant. Explants of different organs in many species develop and regenerate at different rates, and others never grow at all. The haploidity or diploidity of the plantlets produced through tissue culture is also determined by the selection of explant material. Additionally, adopting incorrect transplants raises the chance of microbial infection. Thus, it is essential that you choose an appropriate transplant when commencing tissue culture. There are several reasons for the distinct variations in the capacity for regeneration of diverse organs and explants. The availability of or capacity to transfer endogenous growth regulators, variations in the cell cycle stage, and the metabolic capacities of the cells are among the important variables. The meristematic endpoints of plants, such as the stem tip, auxiliary bud tip, and root tip, are the most often used tissue explants because they have high rates of cell division and either concentrate or synthesise necessary growth-regulating chemicals like auxins and cytokinins. Certain explants, such as the tip of the root, are difficult to separate and contain soil bacteria that cause issues for the tissue culture procedure. Some microflora found in the soil can grow inside roots or create close relationships with them. Because it is challenging to extract soil fragments anchored to roots without harming them, this creates an environment that is favourable to microbial invasion. The tissue culture media will typically be overrun by these related microfloras before plant tissue begins to grow noticeably. Undesirable microbes are also common among aerial (above soil) explants. But gentler rinsing makes it easier to remove them from the explant, and surface sterilisation usually kills the rest. The majority of surface microorganisms fail to form close relationships with plant tissue. Visual examination typically reveals such correlations, such as a mosaic, de-colorization, or localised necrosis on the explant's surface. Acquiring uncontaminated explants can also be accomplished by harvesting seedlings that are aseptically developed from surface-sterilized seedlings. Since seeds have a harder surface and are therefore less susceptible to the infiltration of powerful exterior sterilising chemicals like hypochlorite, the allowable sterilisation parameters for seeds can be far stricter than those for vegetative tissues. The type of explants or the donor plant's physiological stage at the moment of removal determines when culture is initiated. The type of transplant will determine the goal of micropropagation (Yarra *et al*., 2016).

2.1.2 Surface sterilization:

The seedlings of *B. monosperma* were immersed in disinfected water that had been distilled for a whole night after being rinsed under running tap water for fifteen minutes in order to produce explant material. The soaked seeds were cleaned multiple times with autoclaved, deionized water afterwards being surface disinfected for eight minutes in a 1% (v/v) hypochlorite of sodium solution with a couple of drops of Tween-20. (Yarra *et al*., 2017). For the purpose of getting away of any adhering remnants of dust and soil particles, the nodal explant was cut into pieces that were 0.8–1.0 cm long and thoroughly cleaned under flowing water from the tap. The transplanted cells were immersed in 0.2% Bavistin for thirty minutes, cleansed with purified water, and then immersed in 10% 20 for an additional 5 to 10 minutes. Sterile distilled water was then used three to four times to wash the plants. To remove any remaining traces of chemicals from the outermost layer of the nodal explants, the transplants were then cleaned with 0.1% Hgcl2 for 10 minutes and then rinsed again with autoclaved distilled water for 4–5 times (Rathnaprabha *et al*., 2017).

2.1.3. Culture Medium:

The symphony of the culture surroundings constitutes one of the key elements determining the emergence and patterning of plant tissues in culture. Typically, a tissue cultivation medium comprises the following elements: The elements that are needed in concentrations above 0.5 mM/l are known as macronutrients. These comprise the following six major elements, which are found in salt form and make up different media: nitrogen, potassium, phosphorus, magnesium, calcium & sulphur. Typically, the composition of macronutrient stock solutions is approximately ten times that of their ultimate intensity. The elements that require oxygen in quantities less than 0.5 mM/l are known as micronutrients. Iron, Boron, Copper, Manganese, Zinc, Molybdenum, Iodine, Cobalt, and Nickel are among the eight minor elements that are included in this. In most cases, micronutrient solutions for stock are prepared to a hundred times their ultimate strength. Since photosynthesis is impeded in the cultured cells or tissues, carbon must be given to the media in the form of carbs in order for the tissues to proliferate. Sucrose is a common carbon and energy source. The medium's sucrose is quickly transformed into glucose and sucrose. Subsequently, fructose is used after glucose(Rathnaprabha *et al*., 2017). Typically, 2–3% concentrations of sucrose are utilised. Plants need vitamins as catalysts for a variety of metabolic activities. The vitamins that are most commonly utilised in media for culture of cells and tissues include myo-inositol, pyridoxine (B6), nicotinic acid (B3), and thiamine (B1). The range of concentrations is between 0.1 and 10 mgL-1. Although all of the necessary amino acids can generally be synthesised by the cultured cells, some amino acids are able to be supplied to further promote cell development. Amino acid mixes such as casein hydrolysate (0.05–0.1%), glutamine (8 mM L–1), and cysteine (10 mM L–1) are the most often utilised sources of organic nitrogen in culture medium. Positive tissue responses occur when a extensive array of natural extraction are added to the culture media, including the milk from coconuts, extract of yeast, malt extract, potato extract, protein hydrolysis products, powdered fruit such as bananas juice of oranges, and juice from tomatoes. However, protein hydrolysates (0.05–1%) and coconut milk (5–20%) are used to get the desired results. Only two major PGR classes—auxins and cytokinins—are particularly significant in plant tissue culture. those substances that have the ability to gel the medium. Clear gels are produced by gelling agents at comparatively lower concentrations of 1.25–2.5 g/l. These are useful tools for spotting contamination and root development in the culture. Agar, which is agarose as gellan gums, gelrite, etcetera. are examples of common gelling agents(Yarra *et al*., 2016).

The seeds were deposited in MS (Murashige & Skoog, 1962) culture enriched with 3% sucrose (w/v) and 0.8% agar (w/v) (HiMediaR, Mumbai, India) after being thoroughly blot drained on sterile filter paper (Yarra *et al*., 2017). The particles have been washed with SDW four or five times before being thoroughly wiped away on Whatman paper and placed in screw-capped bottles (10 x 8.5 cm) holding 50 ml of two distinct basic inorganic mediums: complete and fifty percent capacity of MS full strength medium (MSF), half (½) strength of MS (MSH) medium, full strength (WPMF) and fifty percent (½) vitality of WPM WPMH) containing 3% sucrose (w/v) and 0.8% (w/v) agar (Himedia, India). For the purpose of in vitro germination of seeds and development of seedlings, all of the abovementioned media were enriched with various amounts of PGRs: thidiazuron (TDZ, 0.45, 2.27, 4.54, and 6.80µM) or N6-benzyladenine (BA, 2.22, 4.40, 6.62, and 8.90 µM) alone.

3. MULTIPLE SHOOT INDUCTION AND ELONGATION

For the reason that of their stubborn nature, plants in the Fabaceae family have been shown to be challenging to regenerate in vitro (Trigiano *et al*., 1992). We first looked at the impact of auxin and cytokinins on direct shoot regeneration from *B. monosperma* var. Lutea cotyledonary nodal transplants in order to create an effective in vitro methodology. The in vitro proliferation of *B. monosperma* was significantly affected by the addition of various quantities of BAP (2.20, 3.11, 4.44, and 6.66 µM) or Kn (2.32, 3.25, 4.65, and 6.97 µM) separately in the culture medium. The BAP concentration alone caused a considerable variation in the rates of shoot induction and multiplication. Compared with either BAP or Kn procedures by itself, 4.44 µM BAP substantially boosted the amount of shoots for each transplant (7.0~ 0.82) and shoot length (3.0~ 0.73) in all of the tested. Kn was added to MS medium, however this did not significantly affect in vitro proliferation. Among the Kn concentrations studied, 3.0~ 0.27 shoots for each transplant with a standard shoot size of 2.8} 0.27 were generated by MS media supplemented with 4.65 µM Kn; nevertheless, the shoot regeneration response is relatively low (20%). The quantity of sprouts for each transplant decreased in media enriched with BAP concentrations either greater or lower than the ideal concentration. Several studies have indicated that BAP works better in various plant species than other cytokinins (Margaret *et al*., 2015; Ramesh *et al*., 2005; Rout *et al*., 2008). The ideal BAP medium did not cause shoots to elongate quickly. In order to assess the shoot elongation, we removed the clumped shoots that had regenerated on the shoot induction medium (SIM), moved them to the shoot elongation medium (SEM), and measured the average shoot length following a period of three weeks of cultivation. The half-MS supplemented with 4.44 µM BAP showed its greatest % shoot extension (77.07} 0.57%) and maximum shoot length (6.92} 0.73 cm). Our findings concur with those of a number of other research projects that documented shoot elongation by the use of BAP (Rasool *et al*., 2009).

For shoot lengthening, the half-strength MS media enriched with BAP performed better than the full-strength MS medium. Following five weeks on the elongation medium, the elongated shoots produced four to five completely extended, healthy leaves (Rathnaprabha *et al*., 2017).

4. IN VITRO ROOTING AND ACCLIMATIZATION

Long shoots (4-6 cm) obtained from cotyledonary nodal explants were divided and cultivated on MS basal medium at full and half strength, with varying IBA concentrations added. On full or half strength MS bottom media without IBA, there was no rooted; however, shoots moved to half-strength medium containing MS enriched with 2.46 µM IBA executed 100% rooting. For the in situ anchoring of *B. monosperma*, half-strength MS medium administered by IBA was superior to full-strength MS medium. Our findings are consistent with those of a number of earlier investigations that found that root induction was facilitated in *Woodfordia fruticosa* (Bulle *et al*., 2012) and *Pterocarpus marsupium* (Tippani *et al*., 2013) when half-strength MS media supplemented with IBA was used. After being moved to pots with sterile soil and a vermiculite (1:1) mixture, the shoots with fully established roots were allowed to harden in the greenhouse. After being moved to the greenhouse, healthy plants were established in less than a month. Excision and inoculation in MS medium gelled with 0.8% (w/v) agar was carried out on strong branches (4.5-5.0 cm) with fully extended leaflets. Within three weeks of culture, roots were started, and the medium was supplemented with 0.5–3.0 mg/L IBA. After the fourth week of culture, the root length and number of each treatment in the rooting experiment were measured. Each treatment included 15 samples (culture tubes), every single having single transplant. After removing the 3- to 4-week-old plantlets from the culture media, the remaining agar residue was carefully rinsed from the roots underneath running water from the faucet. Following their relocation to the pots containing autoclaved organic matter paired with soil (1:1) and sprayed with sterile water, the plantlets were acclimatised before being moved to the greenhouse (Rathnaprabha *et al*., 2017).

5. SOMATIC EMBRYOGENESIS

A popular in vitro technique for plant regeneration, somatic embryogenesis is a crucial biotechnological tool for long-term clonal multiplication. It refers to the procedure by which differentiated embryos are produced commencing somatic cells or tissues. Unlike zygotic embryos, which go via the process of sexual fertilisation, somatic embryos can grow into whole plants. The development of a mass of disorganised cells known as a callus can either directly or indirectly start the somatic embryogenesis process from the explants. Through the creation of embryogenic cultures from zygotic seed, leaf, or stem segments and subsequent embryo multiplication, somatic embryogenesis allows plants to regenerate. After being cultivated for germination and plantlet growth, mature embryos are subsequently placed in soil. A number of plants, primarily trees and foliage plants from various families, have been shown to exhibit somatic embryogenesis. Certain types of cacti have been reported to exhibit the phenomenon (Torres-Munoz & Rodriguez-Garay, 1996). The emergence and growth of somatic embryos in cultured cells are influenced by a number of variables. According to Jayasankar *et al*. (1999), a highly effective technique for somatic embryogenesis on grapevines has been published. This protocol sufficiently demonstrated increased plant regeneration when the tissues were cultivated in liquid media. Plant growth regulators are essential for the renewal and growth of somatic embryos. Breeding nodal stem portions of rose cultivars on media enhanced with different PGRs either separately or in combination generated the highest effectiveness for embryonic callus (Xiangqian *et al*., 2002). When this embryonic callus was cultivated on abscisic acid (ABA) alone, somatic embryos germination rate was high. In addition to being a method of mass plant regeneration, somatic embryogenesis is also thought to be a useful tool for genetic modification. Additionally, the method may be employed to insert genes through genetic transformation (Maynard *et al*., 1998) and generate plants that are resistant to a variety of conditions (Bouquet and Terregrosa, 2003). Han *et al*. (2009) have established an effective technique for the resurrection of cotton varieties that are resistant to Verticillium and Fusarium wilts.

6. ORGANOGENESIS

Organogenesis is a mechanism by which plant parts including roots, branches, and leaves are produced. These organs may arise from undifferentiated cell masses (callus) or straight from the meristem. The process of plant regeneration known as organogenesis involves callingus development and the differentiation of adventitious meristems into organs by varying the concentration of plant growth hormones in the nutritional environment. Skoog and Muller (Skoog and Miller, 1957) were the first to demonstrate that a high auxin to cytokinin ratio induced root regeneration and a high cytokinin to auxin ratio sped up the development of shoots in tobacco callus.

7. GENETIC TRANSFORMATION OF *BUTEA MONOSPERMA* VAR. LUTEA

Tissue culture advancements along with genetic engineering advances—specifically, transformation technologies—have created new avenues for the manufacturing of medications from medicinal plants. To create transgenic plants, it is crucial to regenerate entire plants in vitro from separated cells and tissues. The technology of plant genetic transformation has evolved into a flexible platform for researching plant gene function and improving cultivars. The advancements in tissue culture and plant genetic engineering techniques over many years have culminated in this feat. The creation of *B. monosperma* plants from cotyledonary transplants using an established in vitro regeneration methodology has created a new challenge for the genetic transformation of this significant and priceless medicinal plant. A fast and trustworthy in vitro regeneration strategy is required for the effective genetic transformation of plants. Consequently, this extremely effective and quick regeneration process opens up significant possibilities for *B. monosperma* engineering in order to conduct thorough biomolecular research or to enhance the plant's therapeutic qualities. A vital instrument for researching the molecular underpinnings and directive of metabolic ways is the development of procedures for the effective genetic transformation of medicinal plants. Somaclonal variation restricts the commercial value of plants and is unfavourable in plant regeneration systems and genetic transformation, even though it provides the basis for genetic variety for crop development. Therefore, for a more effective and future-proof application of tissue-culture approaches, it is essential to ascertain the genetic steadiness of the regenerated plants. A popular method for evaluating genetic inconsistencies produced by in vitro methods is the RAPD analysis. Our results corroborate the earlier studies (Pooja *et al*., 2011; Nayak *et al*., 2013; Mahendra *et al*., 2014; Amit *et al*., 2014; Tanvi *et al*., 2015) on the stability of the genome of plants grown in vitro.

8. CRYOPRESERVATION OF CULTURED PLANTS

Plant genetic resources can be stored by cryopreservation at extremely low temperatures, such as liquid nitrogen (LN; -196 °C). It is a technique for preservation that makes it possible to affordably and safely preserve plant genetic resources. Preventing intracellular freezing and inducing the vitrification state in plant cells during cooling in liquid nitrogen are critical for successful cryopreservation. Furthermore, the cryopreservation technique must to be an easy-to-follow routine for everyone. Research on cryopreservation techniques using various plant organs, tissues, and cells dates back to the 1970s. Consequently, various cryopreservation strategies are currently established (e.g., vitrification, dehydration, slow-prefreezing approach). Tropical plant materials, which used to be considered to not be cryopreserved, were also effectively preserved in LN with the advent of such cryopreservation approaches (Bajaj, 1995; Towill & Bajaj, 2002). A novel method for examining genetic stability in cryopreserved plant materials is called cryobionomics (Harding, 2010). According to Corredoira *et al*. (2004), the embryonic tissues can be cryopreserved for later use or to protect germplasm.

Table 2. Technique for Propagation and Conservation of *B. monosperma.*

Stage	Description
Explant preparation	- Seeds undergo surface sterilization and are then germinated in a controlled, sterile environment. - Alternatively, one might collect nodal explants from immature seedlings.
Media selection	The base for both seed germination and shoot multiplication is the Modified Murashige and Skoog (MS) medium. - The intensity of the medium might vary between half and full, depending on the stage.
Shoot multiplication	- Plant growth regulators (PGRs) are incorporated into the MS medium to stimulate bud dormancy release and promote the development of numerous shoots. Benzyl aminopurine (BAP) has demonstrated efficacy in stimulating shoot growth.
Rooting	- When the shoots reach an appropriate size, they are moved to a new MS medium that contains a different plant growth regulator (PGR), such as Indole-3-butyric acid (IBA), in order to promote the development of roots.
Acclimatization	- The rooted plantlets are gradually transitioned from the controlled in vitro environment to a greenhouse setting. - This may entail shifting them to a potting mix and gradually decreasing the level of humidity.
Conservation	- The use of microtubers or cryopreservation techniques can be investigated as methods for storing propagules over a long period of time.

9. Challenges in Propagation and conservation of *B. monosperma*

Butea monosperma, commonly known as the Flame of the Forest or Palash, is a species of flowering tree native to the Indian subcontinent. It is well-known for its vivid orange-red blooms and holds cultural significance in numerous areas (**Fig. 2**). However, *Butea monosperma* confronts a number of difficulties with regard to conservation and propagation, much like many other plant species. Several of these difficulties consist of (Firdaus & Mazumder, 2012; Thirupathaiah, 2007; Erst *et al*., 2015 & Tippani *et al*., 2013).

Butea monosperma seeds have a stiff seed coat that causes uneven and delayed germination, which affects seed viability. To guarantee effective propagation, it is imperative to enhance seed viability and create effective germination techniques (Tandon *et al*., 2003).

Fig. 2. Flowering branch of *Butea monosperma* var. *Lutea* (Source: Aileni *et al*., 2014).

Poor rates of seedling survival: Herbivore grazing, competition from exotic species, and unsuitable habitat conditions all pose problems for *Butea monosperma* seedlings as they try to establish themselves. The survival rates of seedlings can be increased by creating safe growth environments and taking preventative action (Tandon *et al*., 2003 & Aileni *et al*. 2014). Natural habitats are being destroyed and fragmented as a result of agriculture, urbanization, and deforestation (Aileni *et al*. 2014). This presents a serious threat to *Butea monosperma* populations. The trees struggle to sustain healthy populations and procreate as their habitats get smaller.

Overuse: *Butea monosperma* has long been utilized for firewood, lumber, and traditional medicinal uses, among other things. The species' population may diminish as a result of overexploitation of these resources (Reddy *et al*., 2008).

Invasive Species: The growth and regeneration of *Butea monosperma* may be adversely affected by the invasion of non-native plant species. It can be challenging for native species like Butea monosperma to survive when invasive plants compete for resources, change the composition of the soil, and upset natural ecosystems (Kirtikar & Basu, 1935).

Climate Change: *Butea monosperma*'s range and phenology may be impacted by variations in temperature and precipitation patterns linked to climate change. The cycles of fruiting and flowering may be upset by these modifications, which could affect seed distribution and pollination (Erst *et al*., 2015 & Tippani *et al*., 2013). Insufficient Knowledge and Conservation Efforts: Insufficient knowledge regarding the ecological significance of *Butea monosperma* and its current state of conservation can impede efforts towards conservation. It is imperative to raise awareness of the value of protecting this species and its habitat among stakeholders, legislators, and local populations.

10. CONCLUSIONS AND FUTURE PROSPECTS

In vitro cultivation of medicinally significant plants, such as *Butea monosperma* var. lutea, combined with biotechnology seems to offer an almost limitless supply of secondary metabolites with significant therapeutic promise. The main benefit of in vitro propagation for uncommon, sensitive, and severely endangered species is that many plants can be produced from a single explant without eradicating the mother plant. As a result, these can aid in maintaining the diversity of medicinal plants and restoring natural environments. The fundamental tenet of biotechnology is micropropagation because chemical production of the different secondary plant metabolites is not possible. Future generations may benefit from the quick reproduction and long-term usage of medicinal plants made possible by plant cell and tissue culture. This chapter's data and findings are meant to provide light on the prospective use of plant biological technologies in the preservation of the priceless medicinal plant *Butea monosperma*. The biotechnology of *Butea monosperma* is only getting started. It's a good beginning to create methods for tissue culture regeneration of this plant species. Previously, we used cotyledonary nodes from axenic seedlings to establish the in vitro resurrection of this significant plant. Extensive study is urgently needed to establish regeneration methods employing various explants for the preservation and long-term use of this priceless medicinal plant. Transgenic plant development still requires advancements in tissue culture, genetic engineering of *B. monosperma*, and biotechnological applications. In addition to highlighting the connotation of less important metabolites in plants, pharmacologically active chemicals from *B. monosperma* have created new avenues for this tree species' genetic engineering. For this

type of tree, tissue culture offers a viable alternative to produce uncommon and valuable secondary metabolites that are important for medicine. In order to improve secondary metabolites and biomolecule changes, it would be necessary to optimise culture conditions from plant tissue, such as leaf and somatic embryogenesis from various explants that are easily adaptable to tissue culture. Genetic transformation research would also be beneficial.

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