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Research Article

Efficient *In vitro* Propagation Protocol for *Simarouba glauca*: Enhancing Agroforestry, Ecological Restoration, and Sustainable Rural Development

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Abstract

Simarouba glauca predominantly known for its role in oil seed production which is considered as a oil tree with versatile and multipurpose nature that holds immense potential in agroforestry and environmental conservation. Using shoot tips from seedlings grown on Murashige and Skoog (MS) media supplemented with different doses of cytokinins and auxins, the study explores the in vitro propagation of *Simarouba glauca*. The strongest culture response (80.75%) for shoot induction was obtained using MS media with 3.0 mg L^{-1} 6-benzylaminopurine (BAP) and 2.0 mg L^{-1} kinetin; on the other hand, the combination of 3.0 mg L^{-1} BAP and 3.0 mg L^{-1} kinetin produced the greatest number of shoots per explant (4.38). With an average of 4.68 roots per shoot and the best rooting effectiveness (34.50%), indole-3-butyric acid (IBA) at 2.0 mg L^{-1} was the most effective auxin. The plantlets were successfully acclimatized in a controlled environment and are being transferred to the field. The findings demonstrate the potential for large-scale propagation of *Simarouba glauca* to enhance plantation establishment, ecological restoration, and rural livelihoods. This study aims to establish efficient *in vitro* propagation techniques for *Simarouba glauca*, focusing on direct organogenesis from nodal segments. The ultimate goal is to facilitate large-scale production of planting material to support widespread cultivation. By addressing propagation challenges, this research contributes to the development of sustainable *Simarouba glauca* plantations, enhancing ecological restoration, increasing oil production, and improving rural livelihoods.

Keywords: *Simarouba Glauca; In Vitro* Propagation; Ecological Restoration; Agroforestry

Introduction

Simarouba glauca, also called as the paradise tree or oil tree, is a versatile and multi-purpose species that holds immense potential in agroforestry and environmental conservation. Native to Central and South America, this evergreen tree is highly adaptable, thriving in diverse climatic conditions and degraded soils across a wide range of altitudes and temperatures [1]. Its resilience and multifunctionality have made it a subject of global interest, particularly for ecological restoration and sustainable agricultural systems. One of the most significant attributes of *Simarouba glauca* is its ability to produce 2000–2500 kg of oil per hectare annually [2]. The oil, extracted from seeds containing approximately 50% fatty acids, is suitable for human consumption after refining [1]. Beyond its nutritional value, this oil has extensive applications in industries, including the production of soaps, lubricants, paints, and cosmetics [3]. Its role as a renewable and sustainable alternative to petrochemical-based products aligns with global efforts to reduce environmental impact and promote green industries. The

environmental contributions of *Simarouba glauca* are equally significant. Its deep root system effectively prevents soil erosion and enhances soil fertility. Furthermore, its ability to grow on wastelands positions it as an excellent candidate for wasteland reclamation programs [4]. Establishing plantations of this species on degraded soils can restore ecological balance while providing livelihoods for rural communities. As an evergreen species, it also contributes to carbon sequestration, thus supporting global climate change mitigation goals [5]. Despite its advantages, large-scale cultivation of *Simarouba glauca* faces several challenges. The high cost of seeds limits their accessibility to farmers and agroforestry practitioners [1]. Additionally, the species exhibits an unfavorable male-to-female ratio of 3:2, which affects seed production and, consequently, oil yields [6]. These factors highlight the urgent need for innovative propagation techniques to overcome these constraints. In vitro propagation offers a promising solution to address these challenges. Tissue culture methods enable mass multiplication of superior clones, ensuring the production of genetically uniform and high-yielding planting material. Such techniques facilitate rapid multiplication while retaining desirable traits of elite trees. In the case of *Simarouba glauca*, where traditional propagation methods may not suffice, in vitro propagation becomes a critical tool to meet the rising demand for planting material [4]. Although in vitro regeneration protocols have been explored for several agroforestry species, *Simarouba glauca* has received limited research attention. [1] demonstrated regeneration from nodal segments via organogenesis, but systematic studies remain scarce. This research gap underscores the need for developing reproducible in vitro plant regeneration protocols tailored for this species.

Materials and Methods

Shoot tips were collected from eight months old seedlings raised at Forest College and Research Institute, Mettupalayam.

Figure 1: Simarouba glauca shoot tips used for propagation.

After properly cleaning the shoot tips with running tap water, they were cleaned with a liquid soap solution. After that, the shoot tips were washed for six minutes with 0.1% mercuric chloride which acts as a sterilizing agent in the invitro method of propagation. After that, the explants had three thorough washings in a row using distilled water. MS basal medium supplemented with different concentrations and combinations of BAP and kinetin was used to inoculate the explants. The control was the basal MS medium. The various media combinations used in this study were as follows.

- MS (basal)
- MS + BAP (1 mgl-1 to 4 mgl-1)
- MS + Kin (1 mg l-1 to 4 mgl-1)
- MS + BAP + Kin (all the combinations from 1 mg l-1 to 4 mgl-1)

The cultures were subjected to cycles of light (about 2000 lux) and darkness (eight hours and sixteen hours, respectively). The culture was kept at room temperature. The following observations were made.

- Percentage of cultures that induce shoot
- Shoots per explant on average

To induce rhizogenesis, individual shoots were specifically separated from multiple shoots and cultivated on MS medium containing different concentrations of auxins (NAA, IBA, and IAA). Details of the treatment are as follows.

- MS basal
- MS + NAA (0.5 to 2.5 mgl-1)
- MS + IBA (0.5 to 2.5 mgl-1)
- MS + IAA (0.5 to 2.5 mgl-1)

The cultures was exposed to similar conditions extended for shoot induction. These were 16 treatments. Each treatment was replicated five times and the following observations were recorded.

- Percent of rooted shoots
- Average number of roots/shoots

Flowchart for the materials and methods.

This method is a new protocol developed specifically for the efficient *in vitro* Propagation of *Simarouba glauca* developed by Forest College and Research Institute, Mettupalayam.

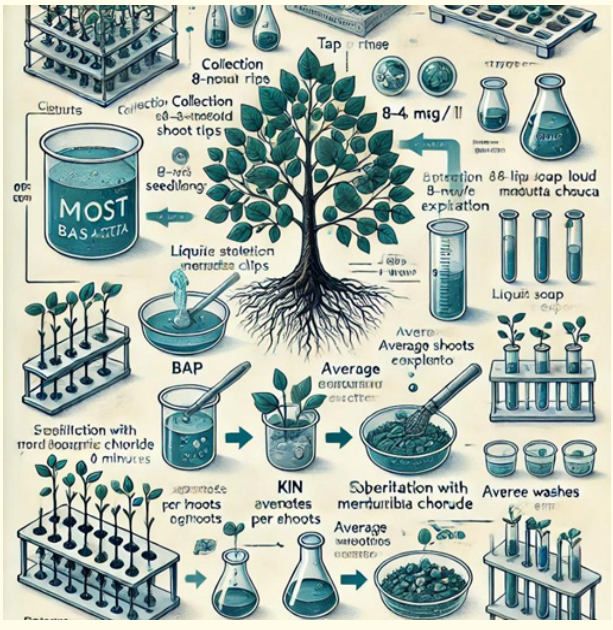

Figure 2

Results and Discussion

Based on the highest values for culture response (80.75%), MS + BAP 3.0 mgl-1 + Kin 2.0 mgl-1 was shown to be the most effective media for inducing shoots from shoot tips. But when compared to MS basal media, MS + BAP 3.0 mgl-1 + Kin 3.0 mgl-1 produced the most shoots per explant (4.38) (Table 1). It was recognized that cytokinins stimulated cell division and performed additional growth-regulating functions [7,8]. A popular natural cytokinin that is frequently utilized in plant tissue culture is kinetin [9,10]. These findings are in consonance with similar reports in Acacia koa [11] and *Acacia albida* [12], which also reported better, results in MS medium supplemented with BAP. Nodal explants of mature trees grown on MS media supplemented with 2.5 mgl-1 of BAP and 0.1 mgl-1 of NAA proved capable of in vitro bud break and shoot multiplication, according to micropropagation investigations conducted on *Simarouba glauca* by [1]. However, MS medium supplemented with kinetin was reported to be ideal for the shoot tip explants derived from seedlings in *Acacia senegal* [13] and in *Sesbania grandiflora* [14]. Auxins are required for rooting of shoots [14]. Of the three auxins tested only IBA reigned supreme with 34.50 per cent rooting. The optimum concentration of these tested was MS + 2.0mgl-1 IBA followed by MS + 1.5 mgl-1 IBA recorded the value of 32.25 per cent (Table 2). The same treatment also proved its superiority with reference to average number of roots per shoot. Many woody plants were known to root when auxins were present [15]. Numerous tree species have demonstrated the beneficial effects of IBA on the roots of in vitro produced plants viz., *Simarouba glauca* [1]; *Albizia procera* [16], *Tectona grandis* [17] *and Zizyphus mauritiana* [18] which are consistent with the result of present findings. The plantlets were removed from culture vessels, washed thoroughly and transplanted into the polbags containing sterile soil, FYM mixture and sand (1:1:1). The plants were kept in a humidified polythene house for better survivality and establishment. Efforts are being made to gradually transfer them to open field.

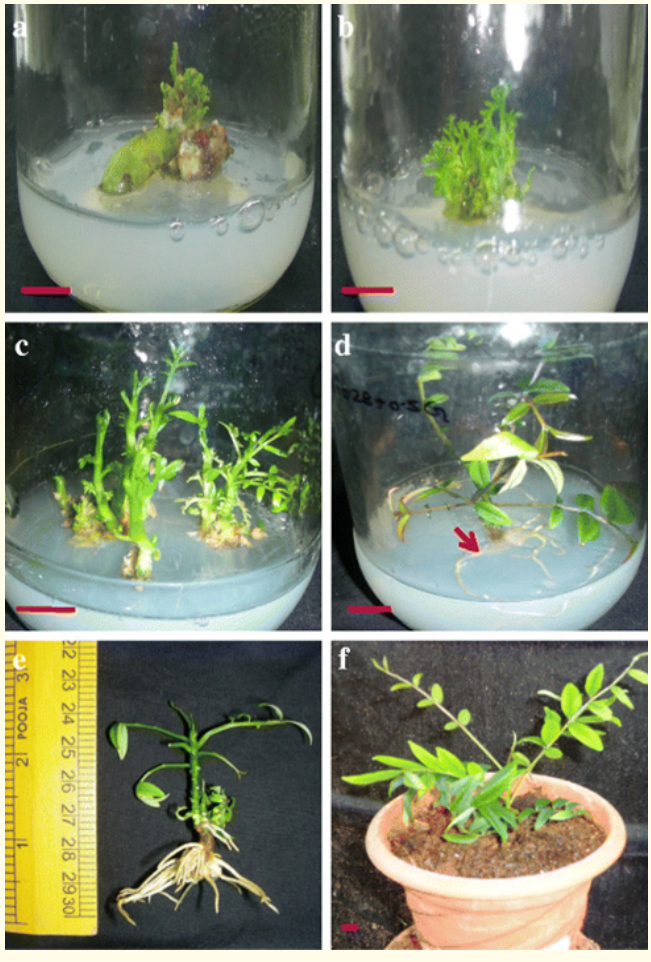

Figure 3: Efficiently propagated *Simarouba glauca* plants after *invitro* propagation

Sl. No.	Treatments	Per cent cultures with shoot induction	Average number of shoots/explant
1	MS basal salt (control)	17.50	1.12
2	MS + BAP 1.0 mgl^{-1}	21.25	1.45
3	MS + BAP 2.0 mgl^{-1}	45.50	1.79
4	MS + BAP 3.0 mgl^{-1}	54.25	2.12
5	MS + BAP 4.0 mgl^{-1}	50.50	1.73
6	MS + Kin 1.0 mgl^{-1}	32.50	1.14
7	MS + Kin 2.0 mgl^{-1}	47.25	1.47
8	MS + Kin 3.0 mgl^{-1}	54.50	1.73
9	MS + Kin 4.0 mgl^{-1}	62.00	1.70
10	MS + BAP 1.0 mgl^{-1} + Kin 1.0 mgl^{-1}	50.25	2.05
11	MS + BAP 1.0 mgl^{-1} + Kin 2.0 mgl^{-1}	44.75	2.56
12	MS + BAP 1.0 mgl^{-1} + Kin 3.0 mgl^{-1}	48.50	2.25
13	MS + BAP 1.0 mgl^{-1} + Kin 4.0 mgl^{-1}	50.25	2.86
14	MS + BAP 2.0 mgl^{-1} + Kin 1.0 mgl^{-1}	53.00	3.01
15	MS + BAP 2.0 mgl^{-1} + Kin 2.0 mgl^{-1}	63.00	3.24
16	MS + BAP 2.0 mgl^{-1} + Kin 3.0 mgl^{-1}	73.25	3.36
17	MS + BAP 2.0 mgl^{-1} + Kin 4.0 mgl^{-1}	64.00	2.84
18	MS + BAP 3.0 mgl^{-1} + Kin 1.0 mgl^{-1}	60.50	2.65
19	MS + BAP 3.0 mgl^{-1} + Kin 2.0 mgl^{-1}	80.75	3.75
20	MS + BAP 3.0 mgl^{-1} + Kin 3.0 mgl^{-1}	58.25	4.38
21	MS + BAP 3.0 mgl^{-1} + Kin 4.0 mgl^{-1}	72.50	3.16
22	MS + BAP 4.0 mgl^{-1} + Kin 1.0 mgl^{-1}	52.00	3.25
23	MS + BAP 4.0 mgl^{-1} + Kin 2.0 mgl^{-1}	50.25	3.33
24	MS + BAP 4.0 mgl^{-1} + Kin 3.0 mgl^{-1}	54.50	3.00
25	MS + BAP 4.0 mgl^{-1} + Kin 4.0 mgl^{-1}	51.75	2.85
	Mean	52.51	2.51
	SEd	2.37	0.29
	CD (0.05)	4.73	0.58

Table 1: Growth regulators' impact on the induction of numerous shoots from shoot tip (seedling) transplant. (Values are means of five replications).

Sl. No.	Treatments	Per cent of rooted shoots	Average number of roots/shoot
1	MS basal salt	0.00	0.00
2	MS + NAA 0.5 mgl^{-1}	0.00	0.00
3	MS + NAA 1.0 mgl^{-1}	0.00	0.00
4	MS + NAA 1.5 mgl^{-1}	11.50	0.00
5	MS + NAA 2.0 mgl^{-1}	12.25	1.65
6	MS + NAA 2.5 mgl^{-1}	14.50	1.24
7	MS + IBA 0.5 mgl^{-1}	0.00	0.00
8	MS + IBA 1.0 mgl^{-1}	19.00	1.60
9	MS + IBA 1.5 mgl^{-1}	32.25	3.94
10	MS + IBA 2.0 mgl^{-1}	34.50	4.68
11	MS + IBA 2.5 mgl^{-1}	16.75	2.24
12	MS + IAA 0.5 mgl^{-1}	0.00	0.00
13	MS + IAA 1.0 mgl^{-1}	0.00	0.00
14	MS + IAA 1.5 mgl^{-1}	0.00	0.00
15	MS + IAA 2.0 mgl^{-1}	0.00	0.00
16	MS + IAA 2.5 mgl^{-1}	0.00	0.00
	Mean	9.38	1.02
	SEd	5.86	0.44
	CD (P=0.05)	11.74	0.87

Table 2: Effect of growth regulators on rooting of micro shoots. (Values are means of five replications).

Conclusion

By establishing an effective protocol for Simarouba glauca in vitro propagation, this study showed that the best conditions for shoot induction are MS medium supplemented with 3.0 mg L^{-1} BAP and 2.0 mg L^{-1} kinetin. The best auxin for rooting was found to be IBA at 2.0 mg L^{-1} which would be helpful in the Sustainable Rural Development as stated in the Sustainable Development Goals.This procedure yielded plantlets that were effectively acclimated, offering a feasible route for widespread dissemination. The described in vitro propagation methods align with sustainability principles by promoting efficient plant production, conserving biodiversity, and supporting livelihoods through agriculture and forestry. By ensuring the scalability and survival of plantlets, the study directly contributes to sustainable natural resource management, climate resilience, and biodiversity conservation; all essential for achieving the SDGs. It helps in achieving SDG 2: Zero hunger; SDG 12: Responsible Consumption and Production; SDG 13: Climate Action; SDG 15: Life on Land and SDG 17: Partnerships for the Goals.

Declarations

There is no conflict of interest between the authors.

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