Full Length Research Article

© 2026, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 17; Issue 03 (May–June 2026); pp 347–351

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Micropropagation of *Oroxylum indicum* (L.) Benth. ex Kurz through Tissue Culture at Similipal Biosphere Reserve, Odisha, India

Rajsekhar Pramanik[1], Nandita Bhakat*[2] and Iswari Prasad Gupta[3]

[1] Assistant Teacher, Department of Biology, Panchkhuri Desh Bandhu High School, Paschim Medinipur - 721 150, West Bengal, India
[2] Assistant Professor, Department of Botany, Narajole Raj College, Paschim Medinipur, West Bengal, India
[3] Associate Professor, University Department of Botany, Dr. Shyama Prasad Mukherjee University, Ranchi, Jharkhand, India

Received: 24 Mar 2026; Revised accepted: 26 May 2026

Abstract

Similipal Biosphere Reserve (SBR) is one of the Sal dominated large forest in state of Odisha. Similipal Biosphere Reserve have 3 protected area- Similipal Tiger Reserve, Kuldiha wildlife Sanctuary and Hadagarh Wildlife Sanctuary. The comparative account of all the sanctuaries, *Oroxylum indicum* depicted the lowest Importance Value Index (IVI) value. The lowest IVI value of that said species is a matter of concern to protect the species in future. The said species is also an ethno-medicinal plant and proper measure should be taken for the propagation and conservation of the species. Naturally, *Oroxylum indicum* reproduces via viable seeds and roots, but the low percentage of seed viability and destructive collection of roots from trees, limits its natural propagation. Propagation of *Oroxylum indicum* through air-layering has been done but due to unavailability of proper plant and branch to air layer become depleted large-scale production is not possible. Foresters should try that process to increase the quantities of this plant *Oroxylum* and conserve this plant from its threat of extinction. In micropropagation study, it was found that the axillary bud showed high frequency of shoot initiation and shoot number at moderate concentration of BAP. Cytokinins have been known to break dormancy of axillary buds resulting in the formation of micro-shoots. Micro-propagation in MS media has been showed very good response.

***Key words*:** Similipal, Kuldiha, Hadagarh, Micropropagation, *Oroxylum indicum*

Oroxylum indicum (L.) Benth. ex Kurz is endangered in Kerala, Maharashtra, Madhya Pradesh and Chhattisgarh, vulnerable in Karnataka and Andhra Pradesh, and likely to become endangered in other states including Odisha and West Bengal soon due to indiscriminate collection, over-exploitation, and uprooting of entire plants bearing roots. Therefore, it is imperative that it be mass-multiplied and conserved in in vitro culture settings. Unconventional methods for improving plants can be found in plant tissue culture. It is now a crucial instrument for mass proliferation and conservation of significant tree species [1]. Normally, *Oroxylum indicum* reproduces by viable seeds and roots; however, its natural propagation is limited by the low proportion of viable seeds and the harmful root harvesting from trees [2]. Therefore, several approaches, such as in vitro procedures, could be applied to proliferate elite genotypes by propagating this plant. Additionally, it has been reported on this tree's in vitro regeneration. They have provided details on the large-scale tissue culture propagation of *Oroxylum indicum* via apical and axillary buds [3]. In vitro micropropagation of *Oroxylum indicum* was developed using nodal explants cultured on MS media supplemented with various combinations of 6-Benzylaminopurine (BAP) and Thidiazuron (TDZ). In vitro shoots were treated with Indole 3- Butyric Acid (IBA) at different doses on half and full strength of MS basal media in order to induce roots [4]. The medium containing IBA showed the greatest root induction [5].

It has been earlier cultivated seedling stem sections of *O. indicum* on Murashige and Skoog (MS) media, either with or without growth regulators [6]. For the growth and proliferation of shoots, use 1-naphthaleneacetic acid and 6-benzylaminopurine. After two weeks of initial culture on all the media supplemented with growth regulators, callus proliferation began [7]. Direct in vitro regeneration of a medicinal tree *O. indicum* through tissue culture was published as a paper. In order to create enormous plantlets, also carried out such kind of in-vitro culture of *O. indicum* in Jabalpur. But in Odisha this type of work should have not been done yet.

The present investigation was therefore undertaken to develop an efficient and reproducible protocol for the in vitro propagation and conservation of *Oroxylum indicum* from the Similipal Biosphere Reserve, Odisha, India, which represents one of the important ecological habitats of this medicinally valuable species. Similipal Biosphere Reserve possesses rich floral diversity and serves as a natural repository of several rare, threatened, and ethnomedicinal plants. However, increasing anthropogenic pressure, habitat destruction, unsustainable harvesting, and low natural regeneration have significantly

***Correspondence to:** Nandita Bhakat, E-mail: nandita@narajolerajcollege.ac.in; Tel: +91 9832153435

Citation: Pramanik R, Bhakat N, Gupta IP. 2026. Micropropagation of *Oroxylum indicum* (L.) Benth. ex Kurz through tissue culture at Similipal Biosphere Reserve, Odisha, India. *Res. Jr. Agril. Sci*. 17(3): 347-351.

reduced the population of *Oroxylum indicum* in this region [8]. Considering its immense medicinal importance in traditional Ayurvedic formulations and indigenous healthcare systems, urgent conservation and propagation strategies are required for sustainable utilization of this species.

Plant tissue culture techniques offer rapid, disease-free, and large-scale multiplication of elite genotypes under controlled environmental conditions [9]. Among the different in vitro approaches, micropropagation through nodal and meristematic explants has gained considerable importance because of its high multiplication rate, genetic uniformity, and year-round production of planting material. Moreover, tissue culture-mediated propagation minimizes the dependence on natural populations and contributes significantly to ex situ conservation programmes for endangered medicinal plants [10]. Previous studies have demonstrated that the application of suitable combinations of cytokinins and auxins plays a vital role in callus induction, shoot proliferation, rooting, and acclimatization of regenerated plantlets [11]. Cytokinins such as 6-Benzylaminopurine (BAP) and Thidiazuron (TDZ) are particularly effective in inducing multiple shoots, while auxins like IBA enhance root initiation and development [12].

In the present study, an attempt was made to standardize an efficient micropropagation protocol for *Oroxylum indicum* using different explants under controlled in vitro conditions. The study focused on optimizing the concentrations and combinations of plant growth regulators for callus induction, shoot multiplication, rooting, and acclimatization of regenerated plantlets. The successful establishment of a reliable regeneration system would not only facilitate rapid clonal multiplication of elite germplasm but also contribute towards the conservation and sustainable management of this endangered medicinal tree species in the Similipal Biosphere Reserve and other forest ecosystems of India.

MATERIALS AND METHODS

Ready-made powder Himedia @ Mumbai was the source of all other add-ons, including the Murashige and Skoog (MS) medium for tissue culture. Each medium received 30 grams per lit of sucrose added as a carbon source. The media's pH was adjusted to 5.8, and it was autoclaved for 20 minutes at 121 °C before 8 grams of agar were added per lit. Plant growth regulators, BAP (1 ppm) and IAA (0.5 ppm) combined, were added to test tube containing sterile culture media (Murashige and Skoog, MS) before the explants were implanted under aseptic conditions. In the same time N/10 Mercuric Chloride (0.1%) solution were used for surface sterilization of plant explants. Also, Suthol (10%) disinfectant was used for sterilization process. A clear test tube measuring 15/125 mm was filled with the medium and autoclaved over a fifteen-minute period at 121°C and 15 psi atmospheric pressure. Before inoculation, the autoclaved medium had been stored at 25°C for a whole day to ensure that there was no visible microbial contamination. Plant growth regulator (PGR) continuous treatment for up to three cultural passes, every lasting 7-10 days, had an effect on direct shoot regeneration. To enable direct multiplication, shoot buds from in vitro-raised shoot were used as the explants for each of the ensuing subcultures. Each experiment was thoroughly randomized and included two or more replications. Every therapy was repeated five times.

RESULTS AND DISCUSSION

Callus proliferation began on all medium supplemented with growth regulators after two weeks of initial culture, reaching its peak in the treatment containing 6-benzyaminopurine 1ppm and Indole-3-Acetic Acid 0.5ppm. Multiple shoot induction occurred during the following four weeks as a result of the subculture of seedling stem sections on the shoot initiation medium. Maximum, on the medium indicated to be optimal for callus proliferation, 90% of explants began shoots, resulting in an average of 10 shoots per explant. Also, nodal meristem showed very good result in culture medium.

Callus initiation was successfully observed in all culture media supplemented with different combinations of plant growth regulators within two weeks of inoculation, indicating the high regenerative potential of explants of *Oroxylum indicum*. However, the degree of callus proliferation varied significantly depending on the hormonal composition of the medium. Among the treatments tested, the medium fortified with 6-benzylaminopurine (BAP) at 1.0 ppm in combination with Indole-3-Acetic Acid (IAA) at 0.5 ppm proved to be the most effective for vigorous callus induction and proliferation. The synergistic interaction between cytokinin and auxin appears to have enhanced cell division and dedifferentiation, resulting in rapid formation of friable and healthy callus tissues. Similar findings have been reported in several medicinal and woody plant species where balanced concentrations of BAP and IAA (Indole-3-acetic acid) promoted efficient morphogenesis and tissue regeneration [13-14].

Subsequent subculturing of seedling stem sections onto shoot initiation medium resulted in multiple shoot induction within four weeks. The highest regeneration response was recorded on the same medium optimized for callus proliferation, where approximately 90% of explants produced shoots with an average of 10 shoots per explant. This high frequency of shoot organogenesis demonstrates the suitability of the hormonal combination for rapid in vitro multiplication of the species. Cytokinins such as 6-benzylaminopurine (BAP) are known to stimulate shoot bud differentiation by promoting active meristematic growth, while low concentrations of auxins support cellular organization and vascular differentiation during organogenesis. The present findings corroborate earlier reports indicating that 6-benzylaminopurine (BAP) in combination with low auxin concentrations significantly enhances shoot multiplication efficiency in endangered medicinal plants [15-16].

The nodal meristem explants also exhibited excellent regenerative responses under the optimized culture conditions. Nodal segments are generally preferred for micropropagation because they contain pre-existing axillary meristems, which minimize somaclonal variation and enhance genetic stability of regenerated plantlets. The superior performance of nodal meristems observed in the present study suggests their suitability for large-scale clonal propagation and conservation of *Oroxylum indicum*. Efficient regeneration through nodal culture has also been emphasized in recent micropropagation studies of threatened medicinal plants owing to its rapid multiplication rate and high survival percentage during acclimatization [17].

The figures further illustrate the morphology and various stages of micropropagation of *Oroxylum indicum*. (Fig 1) represents the characteristic pod morphology of the species, while (Fig 2) depicts the ethnomedicinal plant habit. (Fig 3) demonstrates different laboratory stages of micropropagation including explant establishment, callus formation, shoot induction, and plantlet regeneration under controlled in vitro conditions. These observations confirm the effectiveness of the developed protocol for rapid propagation and conservation of this valuable medicinal species.

Fig 1 Pod of *Oroxylum indicum*

Fig 2 Ethno-medicinal plant *Oroxylum indicum*

Fig 3 Different steps of micropropagation of *Oroxylum indicum* in a laboratory, Ranchi

Micro propagated plants often do not survive without acclimatization or they resume growth only for a few days after soil transfer due to sub optimal conditions during the preceding stages of multiplication, rooting and acclimatization. Successful acclimatization is considered one of the most critical stages in the micropropagation of *Oroxylum indicum* because plantlets developed under in vitro conditions often exhibit poor survival after transfer to ex vitro environments. In the present study, regenerated plantlets initially showed delicate morphology characterized by thin cuticles, poorly developed

vascular systems, reduced epicuticular wax deposition, and non-functional stomata, which are common physiological abnormalities associated with tissue-cultured plants. Such plantlets frequently survive only for a short period after transplantation if adequate hardening procedures are not adopted. The observations recorded during acclimatization demonstrated that gradual exposure to external environmental conditions significantly improved survival and subsequent growth of regenerated plantlets.

Micropropagated plants produced under controlled laboratory conditions are generally exposed to high humidity, low light intensity, constant nutrient supply, and limited gaseous exchange. These conditions induce heterotrophic growth and suppress the development of adaptive features necessary for independent survival under natural conditions. Consequently, sudden transfer of in vitro plantlets to soil often results in excessive transpiration, water stress, chlorosis, and eventual mortality. Similar physiological limitations of tissue-cultured plantlets have been widely reported in medicinal and woody plant species [18-19]. The present findings support the hypothesis that inadequate acclimatization remains one of the major bottlenecks in successful micropropagation protocols.

The different stages of micropropagation illustrated in (Fig 4) demonstrate the sequential development of plantlets from culture initiation to hardening. During the early acclimatization phase, regenerated shoots exhibited reduced vigor immediately after transfer from culture vessels to potting substrates. However, gradual adaptation under high humidity chambers and controlled temperature conditions promoted better root establishment and enhanced shoot elongation. This recovery can be attributed to the progressive restoration of photosynthetic competency and stomatal regulation. Earlier studies have demonstrated that acclimatization treatments involving gradual reduction in humidity and increased light exposure stimulate chloroplast development and improve autotrophic growth capacity in regenerated plants [20].

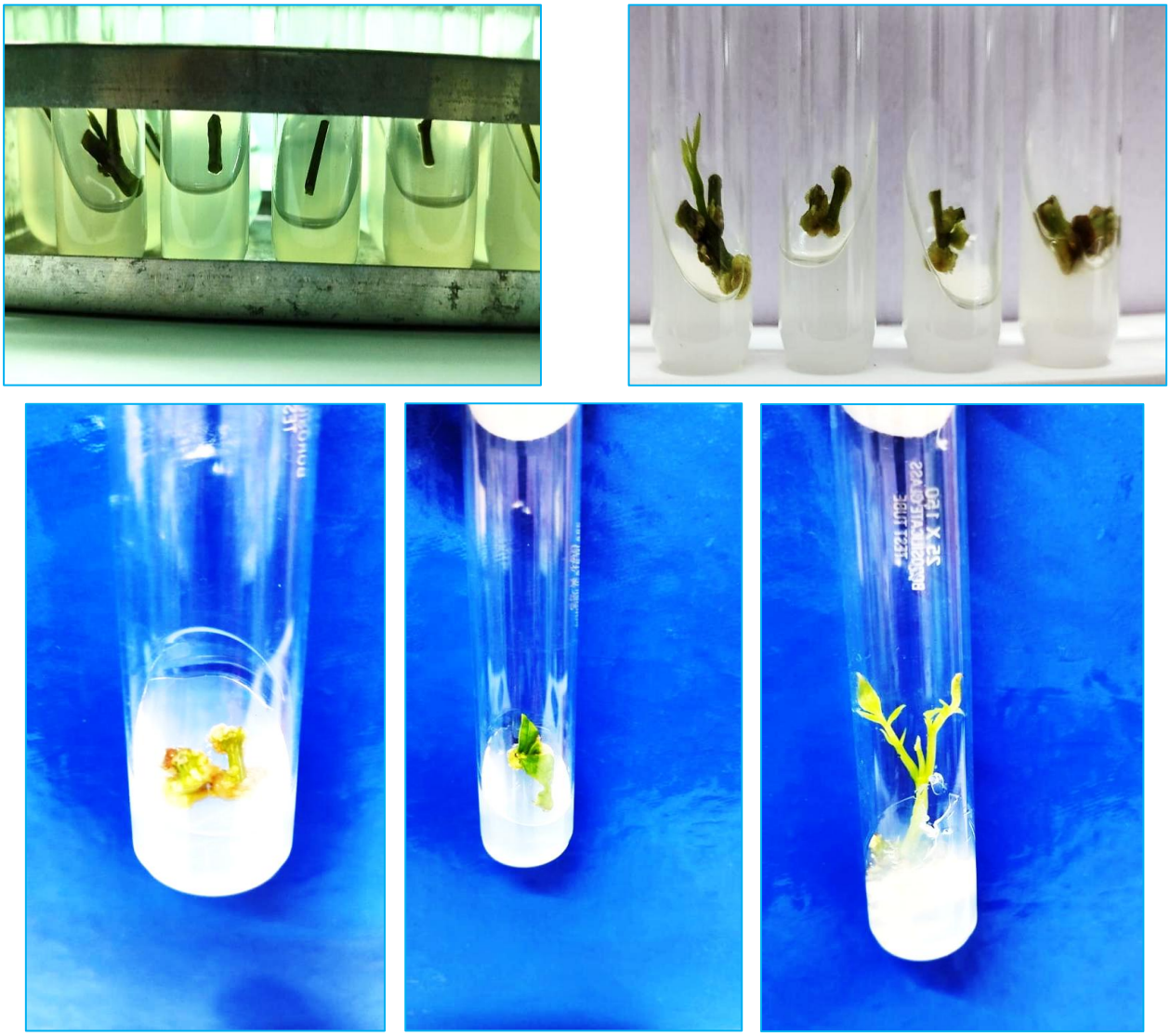

Fig 4 Different stages of micropropagation

The hardening process also plays a crucial role in the anatomical and physiological restructuring of regenerated plantlets. During acclimatization, leaves develop thicker cuticles and functional stomata, while roots become capable of efficient nutrient and water uptake from soil substrates. In the present investigation, plantlets transferred to sterile soil mixtures under controlled greenhouse conditions showed improved survival and active new leaf emergence after acclimatization. Similar improvements in survival percentage during hardening have been reported in other medicinally important species propagated through tissue culture [21]. The successful establishment of hardened plants confirms the efficiency of the developed regeneration protocol for large-scale propagation and conservation of *Oroxylum indicum*.

Acclimatization is particularly important for endangered ethnomedicinal plants because low survival rates after

transplantation can limit the practical application of micropropagation techniques. The present study therefore highlights that optimization of post-culture management practices is equally important as shoot induction and rooting stages. Proper acclimatization not only enhances survival but also ensures vigorous field establishment and long-term conservation of valuable medicinal germplasm. The observations obtained are in agreement with recent reports emphasizing acclimatization as a decisive stage influencing the commercial success of plant tissue culture technology [22].

CONCLUSION

In micropropagation study, it was found that the axillary bud showed high frequency of shoot initiation and shoot number at moderate concentration of BAP. Cytokinins have been known to break dormancy of axillary buds resulting in the formation of micro-shoots. Micro-propagation in MS media has been showed very good response. By this technique we can increase *Oroxylum* and by distributing them to local people in large scale we can conserve this plant by the help of local tribal people. Within this framework, the goal of this work is to multiply the number of *Oroxylum indicum* plantlets by means of tissue culture, direct in-vitro propagation, and seed sowing in various growth regulators (IAA and Cytokinine) and organic growth enhancers (extract of *Cinnamomum*) in a poly house that was built in the SBR. On the other side, make an effort to increase locals' and foresters' knowledge about *Oroxylum indicum*. The precious plant is not something that forest nurseries are interested in growing. It has been proposed that they cultivate that plant in addition to other medicinal and forest plants, and then provide the locals access to the plantlets. Through this mechanism, the plant avoids becoming extinct in the near future. In these ways ethno- conservation of *Oroxylum indicum* along with other ethno-medicinal plants are become possible near future.

LITERATURE CITED

1. Bajpai O, Sahu N, Kumar A, Behera SK, Mishra RM, Chaudhary LB. 2013. Study of plant regeneration potential in tropical moist deciduous forest in Northern India. *International Journal of Environment* 2(1): 153-163.
2. Gokhale M, Bansal YK. 2009. Direct in vitro regeneration of a medicinal tree *Oroxylum indicum* (L.) Vent. through tissue culture. *African Journal of Biotechnology* 8(16): 3777-3781.
3. Dikshit N, Sivaraj N, Dikshit S. 2016. Ethnobotany and traditional health care practices in Similipal Biosphere Reserve, Odisha. *Ecological Environment and Conservation Journal* 22(3): 1191-1197.
4. Saravanan R, Sujana KA, Kannan D. 2019. Phyto-diversity analysis of tree species of Kuldiha Wildlife Sanctuary (KWLS), Odisha, India. *Journal of Applied Life Sciences International* 1: 1-14.
5. Ananthakrishnan R, Ravikumar S, Girija A, Ganapathi A. 2002. In vitro adventitious shoot formation from cotyledon explants of cashew (*Anacardium occidentale* L.). *Scientia Horticulturae* 93(3/4): 343-355.
6. Tiwari SK, Tiwari KP, Siril EA. 2002. An improved micropropagation protocol for teak. *Plant Cell Tissue and Organ Culture* 71: 1-6.
7. Reddy CS, Pattanaik C, Mohapatra A, Biswal AK. 2007. Phytosociological observations on tree diversity of tropical forest of Similipal Biosphere Reserve, Orissa, India. *Taiwania* 52(4): 352-359.
8. Lama R, Manjula BL, Kumbar V, Panda A, Singh A, Kumar S, Marndi S. 2022. Phytochemical screening and antibacterial activities of *Oroxylum indicum* (Linn.): A threatened tree of India. *Asian Pacific Journal of Health Sciences* 9(4S): 140-144.
9. Jayaram K, Prasad MNV. 2008. Genetic diversity in *Oroxylum indicum* (L.) Vent. (Bignoniaceae), a vulnerable medicinal plant by random amplified polymorphic DNA marker. *African Journal of Biotechnology* 7(3): 254-262.
10. Darshan S, Ved DK. 2003. A balanced perspective for management of Indian medicinal plants. *Indian Forester* 129: 275-288.
11. Patel D, Sharma V, Rao M. 2021. Influence of cytokinins and auxins on in vitro shoot regeneration in medicinal plants. *Journal of Applied Biology and Biotechnology* 9(4): 112-118.
12. Parmar VR, Jasrai YT. 2014. Effect of cytokinin and auxin on micropropagation of *Oroxylum indicum* (L.) Vent: A mountain tree of India. *International Journal of Life Sciences Research* 2(4): 58-64.
13. Kumar R, Mishra P, Tiwari S. 2022. Advances in micropropagation and conservation of medicinal plants through tissue culture techniques. *Plant Cell Biotechnology and Molecular Biology* 23(5-6): 45-58.
14. Singh A, Verma R. 2023. Role of plant growth regulators in callus induction and organogenesis of woody medicinal plants. *Plant Tissue Culture and Biotechnology* 33(1): 21-32.
15. Cárdenas-Aquino MdR, Camas-Reyes A, Valencia-Lozano E, López-Sánchez L, Martínez-Antonio A, Cabrera-Ponce JL. 2023. The cytokinins BAP and 2-iP modulate different molecular mechanisms on shoot proliferation and root development in lemongrass (*Cymbopogon citratus*). *Plants* 12(20): 3637. https://doi.org/10.3390/plants12203637
16. Sharma K, Gupta R, Meena M. 2024. Recent trends in micropropagation and in vitro conservation of medicinally important plants. *South African Journal of Botany* 168: 210-221.
17. Rani P, Kumari S, Yadav N. 2023. Nodal explant mediated clonal propagation of threatened medicinal plant species: Recent developments and applications. *Industrial Crops and Products* 198: 116698.
18. Pandey A, Singh R, Kumar V. 2022. Physiological and biochemical changes during acclimatization of tissue culture raised plants. *Plant Cell Biotechnology and Molecular Biology* 23(7/8): 55-67.
19. Sharma P, Kaur H. 2023. Challenges and strategies in acclimatization of micropropagated medicinal plants. *South African Journal of Botany* 156: 412-421.
20. Kumari N, Patel D, Meena R. 2024. Morphological and physiological adaptation of micropropagated plantlets during ex vitro acclimatization. *Plant Cell, Tissue and Organ Culture* 158(2): 245-259.
21. Verma S, Tiwari M, Yadav P. 2021. Hardening and field establishment of in vitro regenerated medicinal plants: Recent advances and future prospects. *Industrial Crops and Products* 170: 113748.
22. Rao K, Sharma V, Gupta S. 2024. Acclimatization and greenhouse establishment of tissue culture regenerated plants: Emerging trends and applications. *Journal of Applied Research on Medicinal and Aromatic Plants* 38: 100542.