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APPENDIX-A

Table 9.1: Showing different nutrient compositions used in MS media

Macronutrients	**Chemical formula**	**g/L (Concentration)**
Ammonium nitrate	NH_4NO_3	16.5
Potassium nitrate	KNO_3	19
Calcium chloride	$CaCl_2.2H_2O$	4.4
Magnesium sulphate	$MnSO_4.4H_2O$	3.7
Potassium dihydrogen orthophosphate	KH_2PO_4	1.7
Micronutrients		
Manganese sulphate	$MnSO_4.\ 5H_2O$	2.23
Zinc sulphate	$ZnSO_4.7H_2O$	0.86
Potassium iodide	KI	0.086
Cupric sulphate	$CuSO_45H_2O$	0.0026
Sodium molybdate	$Na_2MoO_4.2H_2O$	0.025
Cobalt chloride	$CoCl_2.6H_2O$	0.0026
Boric acid	H_3BO_3	0.62
Vitamins		
Nicotinic acid	$C_6H_5NO_2$	0.05
Thiamine hydrochloride	$C_{12}H_{17}ClN_4OS.HCl$	0.01
Pyridoxine hydrochloride	$C_8H_{12}N_2O_2.2HCl$	0.05
Glycine	$C_2H_5NO_2$	0.2
Iron source		
Sodium EDTA	$C_{10}H_{14}N_2O_8Na2H_2O$	2.78
Ferrous sulphate	$FeSO_4.7H_2O$	3.72
Myo-inositol		0.1g
Sucrose		30g
Phytagel		2g

APPENDIX-B

B: Chemicals Used

Acetic acid

Acetone

Agar agar

Agarose

Ammonium nitrate

Ascorbic acid

Bavistin

Boric acid

Calcium chloride

Cobalt chloride

CTAB buffer

De-ionized water

Disodium molybdate

DPPH

Ethanol

Ethidium bromide

FCR

Ferrous sulphate

Gallic acid

Glycine

Magnesium chloride

Manganese sulphate

Mercuric chloride

Methanol

MS media

Myo-inositol

Nicotinic acid

Nucleotide

PCR master mix

Phytagel

Potassium dihydrogen phosphate

Potassium nitrate

Primer

Pyridoxine hydrochloride

Quercetin

Sodium EDTA

Sucrose

Taq Polymerase

Thiamine hydrochloride

APPENDIX C

C: Instruments used

Autoclave (Optics technology)

Laminar air flow (Optics technology)

Growth chamber (Bhanu Biotech)

Thermal cycler (Applied Biosystem)

HPLC (waters)

Magnetic stirrer (optics technology)

Lyophilizer (Telstar lyoquest freeze dryer)

Weighing balance

Incubator (Optics technology)

Hot air oven (Optics technology)

Centrifuge (RV/FM, super spin, plastocraft, India)

Deep freezer -80^{o}C (Blue star, CRESCENT)

Water bath incubator shaker (Remi Model No. KWBS-2)

Refrigerator (Godrej)

Mili Q (Ellix)

Micropipette (Eppendrof)

pH meter (Elico pH meter LI617)

APPENDIX D

D: Abbreviations

MS: Murashige and Skoog

IAA: Indole-3-acetic acid

IBA: indole-3-butyric acid

NAA: 1-naphthaleneacetic acid

2,4-D: 2,4-diclorophenoxyacetic acid

BAP: 6-benzylaminopurine

GA_3: gibberellic acid

SE: standard error

ANOVA: Analysis of variance

BM: Basal media

RAPD: Random amplified polymorphic DNA

GAE: Gallic acid equivalent

QE: Quercetin equivalent

AAE: ascorbic acid equivalent

HPLC: high pressure liquid chromatography

RP: Reverse phase

GC-MS: gas chromatography-mass spectroscopy

NMRC: nuclear magnetic resonance cryoporometry

PSD: pore size distribution

FCR: folin-ciocalteu reagent

DPPH: 2,2-diphenylpicrylhydrazyl

RT: retention time

TDZ: Thidiazuron

EtBr: Ethidium bromide

F: Research articles published in Scopus and Web of science

Research article I

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RESEARCH ARTICLE

In vitro propagation and plant regeneration of *Torenia crustacea* (L.) Charm. & Schltdl; an important ethnic medicinal plant

Tikendrajit Baro[1] & Sandeep Das[*]

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Baro T, Das S. *In vitro* propagation and plant regeneration of *Torenia crustacea* (L.) Charm. & Schltdl; an important ethnic medicinal plant. Plant Science Today. 2022; 9(sp2): 30–35. https://doi.org/10.14719/pst.1708

Abstract

Torenia crustacea (L.) Cham. & Schltdl is an important medicinal herb used in India, Indonesia and Malaysia. This herb is used by Bodo tribes in the Bodoland Territorial Region, Assam to treat various diseases like diabetes. The present experiment developed an efficient protocol for *in vitro* mass propagation technique for *Torenia crustacea* using its nodal explants. For tissue culture, the rapidly growing nodal explants of *Torenia crustacea* were used. Effective explant surface sterilisation was found at 15 min. of treatment with 2% sodium hypochlorite resulting in a maximum explant survival rate and a lower rate of explant contamination after 21 days of explant culture. Media containing full strength MS (Murashige and Skoog's) + BAP (6- Benzyl amino purine) (1 mg/l) were found most effective for the establishment of the explant. The highest shoot proliferation and multiplication were observed in the media containing full strength MS + BAP (1 mg/l) + NAA (Naphthalene acetic acid) (0.2 mg/l); in this combination, an average of 16 shoots formed per nodal explant. The rooting of explants was observed highest in MS media along with 0.4 mg/l concentration of NAA and 1 mg/l BA. The *in vitro* multiplied shoots were grown in plastic pots containing vermicompost fertiliser and soil mixture and successfully grown in the open field condition.

Keywords

Explant culture, MS media, shoot multiplication, surface sterilisation, tissue culture

Introduction

Numerous traditional medicinal plants exist in nature in different regions of India. The North-Eastern states of India are one of the richest areas of medicinal and aromatic plant resources in the world (1) and most of the medicinal plants are unexplored. Due to the high demands of crude drugs, the medicinal plants are being overexploited, resulting in the threat for many rare species and many important plants are disappearing from nature (2). Because of various anthropogenic activities, many species are on the way to extinction without being properly explored or identified which could have immense medicinal property. Hence, identifying and preserving these plants are very important as they are widely used by people. Plant tissue culture is one of the fundamental tools for plant science and is continuously employed in mass propagation, plant improvement and conservation of plant species (3). Plant tissue culture or micropropagation is one of those promising techniques for the rapid growth of the plant tissue and plant organ under sterile conditions in a culture medium. The propagation is uni-

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RESEARCH ARTICLE

In vitro propagation and somaclonal variation study of *Phlogacanthus thyrsiformis* Nees an ethnic medicinal plant

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Abstract

Bodoland Territorial Region is very rich in natural bioresources, and the Bodo tribes of the region use numerous ethnic medicinal plants to treat different kinds of diseases. *Phlogacanthus thyrsiformis* Nees is an important medicinal shrub species used in the region to treat different kinds of diseases. The objectives of the present study were to develop an efficient *in vitro* mass propagation technique of the species using nodal explant and study genetic stability in the genome of *in vitro* propagated plantlets by different RAPD markers. Effective explant surface sterilisation resulted at 2 min of treatment with 0.1% mercuric chloride. Explant responses were found most effective in the full strength MS + 1mg/l BAP (6- benzyl amino purine), and explant highest shoot proliferation multiplication and rooting were found in the media MS + 1 mg/l BAP + 0.250 mg/l NAA. *Ex vitro* rooting of micro propagated plants was most effective when the explants were dipped in 1 mg/l IBA for an hr. RAPD assays were conducted using eight sets of random primers (OPC02, OPC05, OPC07, OPC08, OPC09, OPX06, DK2 and OPA01). All the primers except OPC07 and OPA01 formed monomorphic DNA bands in gel electrophoresis and polymorphism was detected by OPC07 and OPA01 primer.

Keywords

explants culture, molecular marker, MS media, plant regeneration, tissue culture, RAPD

Introduction

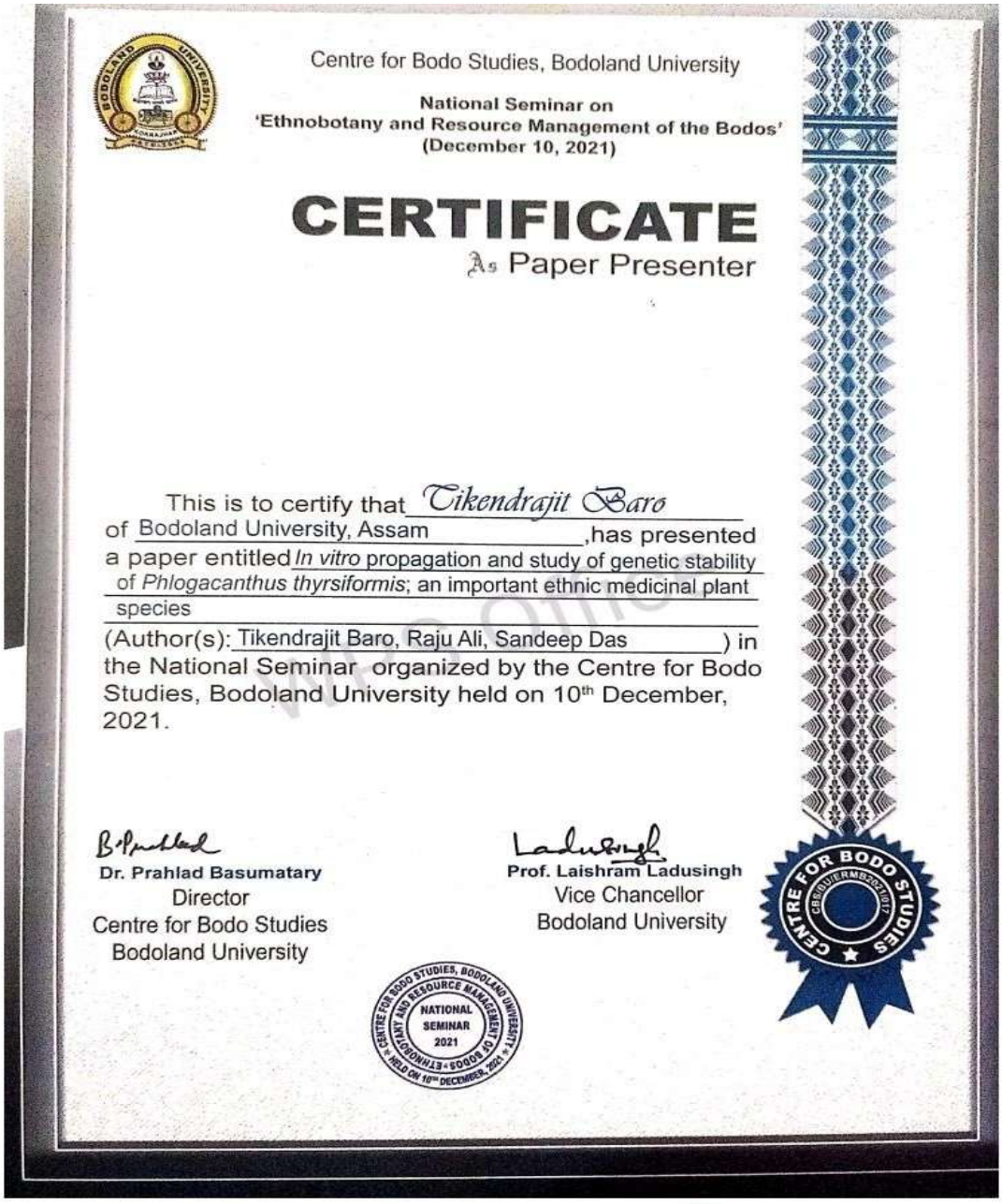
The global population mostly relies on ethnic medicine (about 80% of the total population) for primary healthcare, most of which is taken from plant extracts (1). Traditional medicines play a crucial role in the health care and treatment of diseases in most developing countries (2). *Phlogacanthus thyrsiformis* Nees (Barsukha) is a shrub species of the Acanthaceae family. It is 3-7 feet in height. It is used as folk medicine in Assam and its distribution ranges from Bhutan to the North-Eastern states of India in the Himalayas and Indo-China, southern China and Sulawesi (3). The shrub is used to treat allergies, cough, cold, chronic bronchitis, rheumatoid arthritis, asthma etc. also the leaf extract is used as anti-bacterial and also effective in inhibiting the growth of HeLa cells (3). From the survey, it was found that the flowers of the plant are also consumed by local peoples considering medicinal importances like- anti gastric, anti- diabetes, anti-cough to treat cold fever.

Because of the presence of bioactive potential in medicinal plants, their demands are being higher day after day. Further, due to deforestation,

APPENDIX G

G: Paper presentation in international and national seminar

Paper presented in National seminar entitled "*in vitro* propagation and study of genetic stability of *Phlogacanthus thyrsiformis;* an important medicinal plant species'



Centre for Bodo Studies, Bodoland University

National Seminar on
'Ethnobotany and Resource Management of the Bodos'
(December 10, 2021)

CERTIFICATE

As Paper Presenter

This is to certify that Tikendrajit Baro
of Bodoland University, Assam, has presented a paper entitled *In vitro* propagation and study of genetic stability of *Phlogacanthus thyrsiformis*; an important ethnic medicinal plant species
(Author(s): Tikendrajit Baro, Raju Ali, Sandeep Das) in the National Seminar organized by the Centre for Bodo Studies, Bodoland University held on 10th December, 2021.

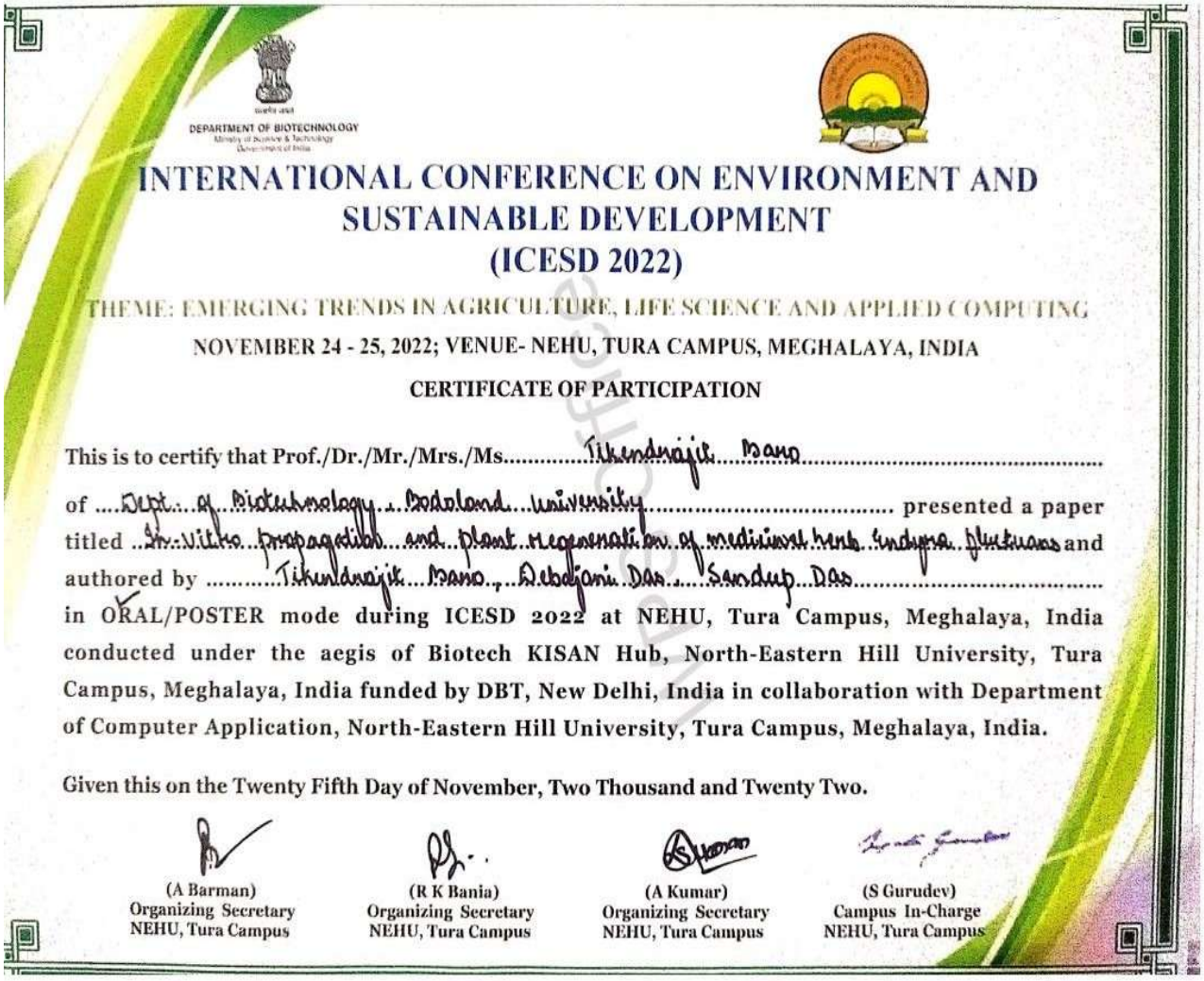
Dr. Prahlad Basumatary
Director
Centre for Bodo Studies
Bodoland University

Prof. Laishram Ladusingh
Vice Chancellor
Bodoland University

CENTRE FOR BODO STUDIES

CENTRE FOR BODO STUDIES, BODOLAND UNIVERSITY * ETHNOBOTANY AND RESOURCE MANAGEMENT OF BODOS * HELD ON 10TH DECEMBER, 2021 * NATIONAL SEMINAR 2021

Paper presented in international conference entitled " *In vitro* propagation and plant regeneration of medicinal herb *Enydra fluctuans*"



DEPARTMENT OF BIOTECHNOLOGY

INTERNATIONAL CONFERENCE ON ENVIRONMENT AND SUSTAINABLE DEVELOPMENT (ICESD 2022)

THEME: EMERGING TRENDS IN AGRICULTURE, LIFE SCIENCE AND APPLIED COMPUTING

NOVEMBER 24 - 25, 2022; VENUE- NEHU, TURA CAMPUS, MEGHALAYA, INDIA

CERTIFICATE OF PARTICIPATION

This is to certify that Prof./Dr./Mr./Mrs./Ms. Tikendrajit Mano

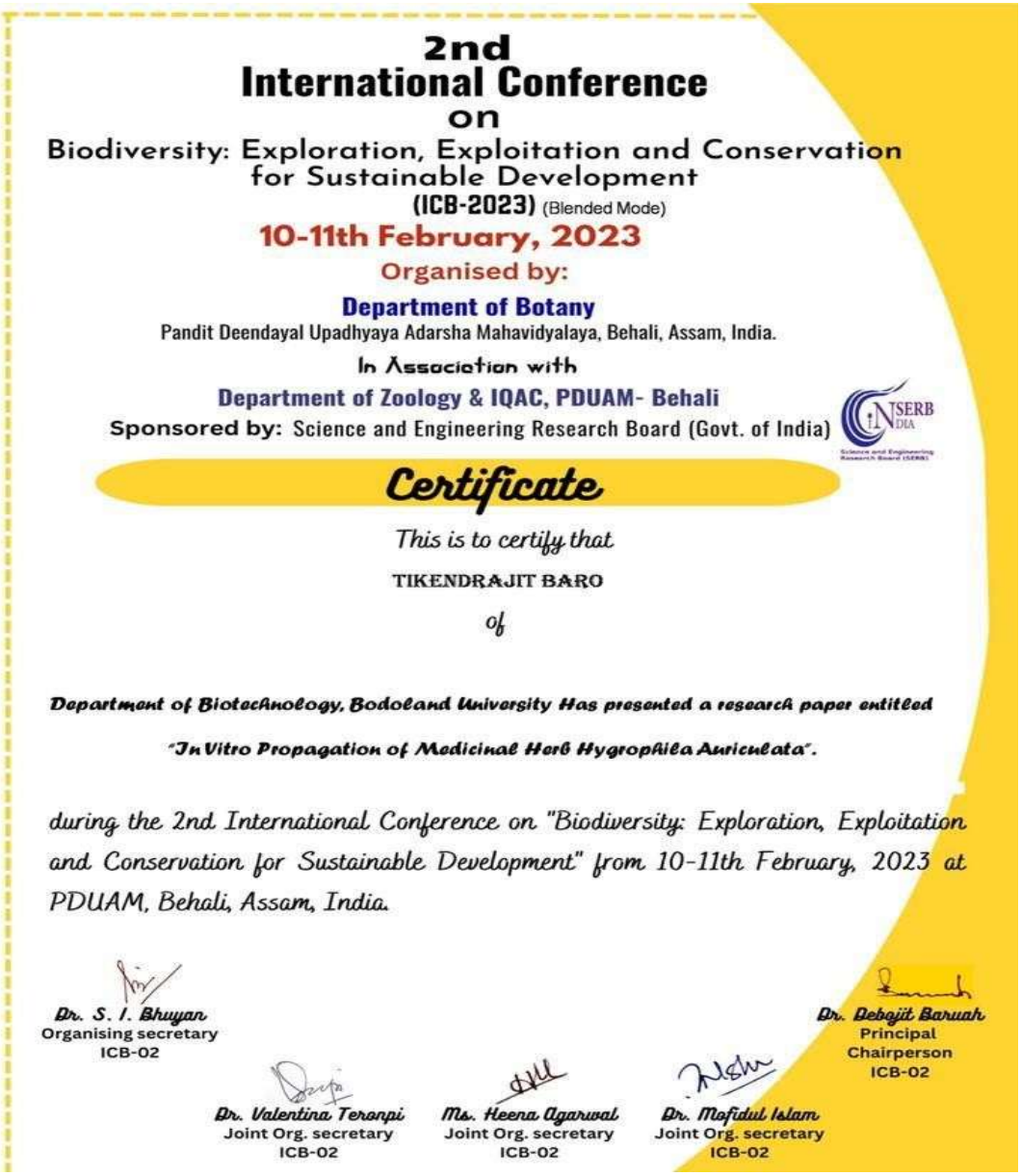
of Dept. of Biotechnology, Bodoland University presented a paper titled In-Vitro propagation and plant regeneration of medicinal herb Enydra fluctuans and authored by Tikendrajit Mano, Debajani Das, Sandup Das

in ORAL/POSTER mode during ICESD 2022 at NEHU, Tura Campus, Meghalaya, India conducted under the aegis of Biotech KISAN Hub, North-Eastern Hill University, Tura Campus, Meghalaya, India funded by DBT, New Delhi, India in collaboration with Department of Computer Application, North-Eastern Hill University, Tura Campus, Meghalaya, India.

Given this on the Twenty Fifth Day of November, Two Thousand and Twenty Two.

(A Barman)	(R K Bania)	(A Kumar)	(S Gurudev)
Organizing Secretary	Organizing Secretary	Organizing Secretary	Campus In-Charge
NEHU, Tura Campus	NEHU, Tura Campus	NEHU, Tura Campus	NEHU, Tura Campus

Paper presented in International conference entitled " *in vitro* propagation of medicinal herb *Hygrophila auriculata*"



2nd
International Conference
on
Biodiversity: Exploration, Exploitation and Conservation for Sustainable Development
(ICB-2023) (Blended Mode)
10-11th February, 2023
Organised by:
Department of Botany
Pandit Deendayal Upadhyaya Adarsha Mahavidyalaya, Behali, Assam, India.
In Association with
Department of Zoology & IQAC, PDUAM- Behali
Sponsored by: Science and Engineering Research Board (Govt. of India)

Certificate

This is to certify that

TIKENDRAJIT BARO

of

Department of Biotechnology, Bodoland University Has presented a research paper entitled

"In Vitro Propagation of Medicinal Herb Hygrophila Auriculata".

during the 2nd International Conference on "Biodiversity: Exploration, Exploitation and Conservation for Sustainable Development" from 10-11th February, 2023 at PDUAM, Behali, Assam, India.

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