

International Journal for Biotechnology and Molecular Biology Research

Volume 4 Number 6 October 2013

ISSN 2141-2154



*Academic
Journals*

ABOUT IJBMBR

The **International Journal for Biotechnology and Molecular Biology Research (IJBMBR)** (ISSN 2141-2154) is published Monthly (one volume per year) by Academic Journals.

International Journal for Biotechnology and Molecular Biology Research (IJBMBR) provides rapid publication (monthly) of articles in all areas of the subject such as Green energy from chemicals and bio-wastes, Studies in the graft copolymerization of acrylonitrile onto cassava starch by ceric ion induced initiation, Antimutagenic activity of aqueous extract of Momordica charantia, Ethnomedicinal plants and other natural products with anti-HIV active compounds and their putative modes of action etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in IJBMBR are peer-reviewed.

Submission of Manuscript

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author

[Click here to Submit manuscripts online](#)

If you have any difficulty using the online submission system, kindly submit via this email ijbmb@academicjournals.org.

With questions or concerns, please contact the Editorial Office at ijbmb@academicjournals.org.

Editors

Prof Atagana, Harrison

*Institute for Science and Technology Education
University of South Africa*

Prof. UC Banerjee

*Department of Pharmaceutical Technology
(Biotechnology)
National Institute of Pharmaceutical Education and
Research
Punjab, INDIA*

Dr. Y. Omid

*Faculty of Pharmacy,
Research Center for Pharmaceutical Nanotechnology,
School of Advanced Biomedical Sciences,
Tabriz University of Medical Sciences,
Tabriz, Iran.*

Prof. Mohamed E. Wagih

*University of New Brunswick (UNB-SJ),
Saint John College, NB,
E2L 4L5, Canada*

Dr. Sripada M. Udupa

*ICARDA-INRA Cooperative Research Project,
International Center for Agricultural Research in the
Dry Areas(ICARDA), B.P. 6299,
Rabat Instituts, Rabat, Morocco*

Dr. Amjad Masood Husaini

*Sher-e-Kashmir University of Agricultural Sciences &
Technology
Bohlochipora, Dr. Ali Jan Road,
Nowshera, Srinagar, J&K-190011, India*

Dr. Om Prakash Gupta

*Directorate of Wheat Research (ICAR)
Post Box-158, A
grasain Marg, Karnal-132001,
Haryana, India*

Editorial Board

Dr. Amro Hanora

*Suez Canal University, Department of Microbiology
and Immunology,
Faculty of Pharmacy, Suez Canal University,
Box 41522 Ismailia, Egypt*

Dr. C. Rajasekaran

*VIT University
School of Bio-Sciences & Technology (SBST)*

Dr. Yasar Karadag

*Gaziosmanpasa University
Faculty of Agriculture,
Department of Field Crops, Tokat-Turkey*

Dr. Ahmet Tutus

*KSU (Kahramanmaras Sutcu Imam University)
Faculty of Forestry,
Department of Forest Industrial Engineering,
Kahramanmaras 46100 Turkey*

Dr. Vinod Joshi

*Desert Medicine Research Centre,
Indian Council of Medical Research
New Pali Road, Jodhpur, India*

Dr. Eshrat Gharaei Fathabad

*K.M.18 Khazarabad road,
Sari, Mazandaran, Iran*

Dr. Shashideep Singhal

*121 Dekalb Ave, Brooklyn,
NY 11201, New York, USA*

Dr Masayoshi Yamaguchi

*101 Woodruff Circle, 1305 WMRB,
Atlanta, Georgia 30322-0001, USA*

Dr. Okonko Iheanyi Omezuruike

*Department of Virology,
Faculty of Basic Medical Sciences,
College of Medicine,
University College Hospital,
Ibadan, Nigeria*

Dr. S. M. Shahid

*University of Karachi,
Karachi-75270, Pakistan*

Prof. Reda Helmy Sammour

*Botany Department,
Faculty of Science,
Tanta University,
Tanta, Egypt*

Dr. Premendra D. Dwivedi

*Food Toxicology Division,
Room No 303,
P.O. Box 80,
M. G. Road, Lucknow-226001, UP, India*

Dr. Amro Hanora

*Microbiology and Immunology department,
Faculty of Pharmacy,
Suez Canal University,
Box 41522 Ismailia, Egypt*

Dr. Tamilnadu

*1501 N. Campbell Ave
Tucson, AZ 85724 India*

Dr. Yadollah Omid

*Faculty of Pharmacy,
Tabriz University of Medical Sciences,
Daneshgah St., Tabriz, Iran*

Dr. Mohsen Selim Asker

*National Research Centre, Dokki, Cairo,
Egypt*

Dr. Fanuel Lampiao

P.O.Box 360, Blantyre, Malawi

Prof. Mohamed E. Wagih

Saint John, NB, E2L 4L5, Canada

Dr. Santosh Kumar Singh

*Centre of Experimental Medicine and Surgery,
Institute of Medical Sciences,
Banaras Hindu University, Varanasi-221005, India*

Dr. Zhanhuan Shang

*No.768, Jiayuguan West Road,
Lanzhou City, Gansu Province, China*

Dr. Worlanyo Eric Gato

*Southern Illinois University – Carbondale,
1245 Lincoln Dr, 144 Neckers, Carbondale IL 62901*

Dr. Chun Chen

*College of Life Sciences,
China Jiliang University
Xueyuan Street, Xiasha, Hangzhou,
Zhejiang Province, PR China*

Dr. Efthimios J.Karadimas

*LGI, Leeds NHS Trust 10th Timoleonos Vassou str,
11521, Athens Greece*

Dr. Samuel Toba Anjorin

University of Abuja, Abuja, Nigeria

Dr. Rupali Agnihotri

*Department of Periodontics,
Manipal college of Dental Sciences,
Manipal,576104. Karnataka. India*

Dr. Mahbuba Begum

*Tuber Crops Research Centre,
Joydebpur, Gajipur-1701, Bangladesh*

Prof. S. Mohan Karuppayil

*School of Life Sciences
Srtm University
Nanded. MS. India*

Dr. Neveen Helmy Aboelsoud

*Complementary Medicine Researches and Application
Department
National Research Center, Cairo
Egypt.*

Dr. D.E. Chandrashekar Rao

*National Research Council,
Plant Biotechnology Institute
Canada*

Dr. Nikolaos Papanas

*Democritus University of Thrace
G. Kondyli 22, Alexandroupolis, Greece*

Dr. Sivakumar Swaminathan

*Iowa State University
USA*

Dr. El Sayed Hussein El Sayed Ziedan

*National Research Centre,
Plant Pathology Department
Tahrir St.,Dokki Cairo,
Egypt*

Dr. Chethan Kumar M

*Post Graduate Departments of Bio-technology and Biochemistry,
Ooty Road, Mysore - 570 025,
Karnataka,
India*

Dr. M. Sattari

*Rice Research Ins. of Iran
Iran*

Dr. Zaved Ahmed Khan

*VIT University
India*

Dr. Subbiah Poopathi

*Vector Control Research Centre
Indian Council of Medical Research
(Ministry of Health & Family Welfare,
Govt. of India)
Medical Complex, Indira Nagar
India*

Dr. Reyazul Rouf Mir

*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT),
Patancheru - 502 324, Greater Hyderabad,
India*

Dr. Prasanna Kumar S

*Virginia Commonwealth University,
USA*

Dr. Naseem Ahmad

*Plant Biotechnology Laboratory
Department of Botany
Aligarh Muslim University
Aligarh- 202 002, (UP)
India*

Dr. Zhen-Xing Tang

*Food Bioengineering institute, Hangzhou Wahaha Co.
Ltd, Hangzhou,
Zhejiang,
China*

Dr. Jayanthi Abraham

*VIT (Vellore Institute of Technology) University,
Tamilnadu,
India*

Dr. Gobianand Kuppannan

*National Institute of Animal Science
South Korea*

Dr. R. Harikrishnan

*Jeju National University
South Korea*

Dr. Asit Ranjan Ghosh

*Vellore Institute of Technology (VIT) University,
School of Bio Sciences & Technology,
Medical Biotechnology Division, Vellore-632014,
India*

Dr. Kamal Dev

*Shoolini University of Biotechnology and Management
Sciences (SUBMS)
India*

Dr. Wichian Sittiprapaporn

*Maharakham University
Thailand*

Dr. Vijai Kumar Gupta

*Molecular Glycobiotechnology Group, Department of
Biochemistry,
School of Natural Sciences,
National University of Ireland, Galway,
Ireland*

Dr. Jeffy George

*Department of Microbiology and Immunology
F. Edward Hébert School of Medicine
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road,
Bethesda, MD 20814
USA.*

Dr. Gyanendra Singh

*Stanley S. Scott Cancer Center,
School of Medicine,
Louisiana State University Health Sciences Center
New Orleans, LA 70112,
USA.*

Dr. Anupreet Kour

*1620 Chevy Chase Dr.
Champaign, IL 61821
USA.*

Dr. Arun Sharma

*Institute for Plant Genomics and Biotechnology (IPGB)
Borlaug Center,
TAMU 2123
Texas A&M University
College Station, TX 77843
USA.*

Dr. Mohsen Asker

*Microbial Biotechnology Dept.
National Research Centre
Cairo,
Egypt.*

Dr. Elijah Miinda Ateka

*Department of Horticulture,
Jomo Kenyatta University of Agriculture and
Technology (JKUAT)
Kenya.*

Dr. Jozélio Freire De Carvalho

*Faculdade de Medicina Da USP, Reumatologia
Av. Dr. Arnaldo, 455 - 3º andar – Sala 3133.
São Paulo - SP
Brazil*

Dr. Premendra Dhar Dwivedi

*Food Toxicology Division
Industrial Institute of Toxicology Research,
Post Box No: 80, Mahatma Gandhi Marg,
Lucknow 226001,
India*

Dr. Muhammad Abd El-Moez El-Saadani

*Universities and Research Center District,
New Borg El-Arab,
P.O.Box: 21934 Alexandria,
Egypt.*

Dr. Donald J. Ferguson

*Advanced Orthodontic Training Program,
Nicolas & Asp University College
Dubai,
UAE*

Dr. Kalyan Goswami

*Department of Biochemistry & JB Tropical Disease
Research Centre,
Mahatma Gandhi Institute of Medical Sciences,
Sevagram, Wardha-442102*

Dr. A.K. Handa

*National Research Centre for Agroforestry,
Gwalior Road, JHANSI-284003 UP
India.*

Dr. Amjad M.Husaini

*Metabolic Engineering & Biotechnology Laboratory
Division of Plant Breeding & Genetics
Sher-e-Kashmir University of Agricultural Sciences &
Technology of Kashmir
J&K-191121,
India*

Dr. Vinod Joshi

*Laboratory of Virology & Molecular Biology,
Desert Medicine Research Centre,
Pali Road, Jodhpur-342 005,
India*

Dr. T. Kalaivani

*D/O S. Thiagarajan
B-43, Rajaram Nagar,
Salem - 636 007,
Tamil Nadu, India*

Dr. Priya Kalia

*Orthopaedic Research Unit,
Department of Surgery,
Cambridge University, Cambridge,
UK*

Dr. Patricia Khashayar

*Tehran University of Medical Sciences
Endocrinology and Metabolism Research Center
Shariati Hospital*

Dr. Zaringhalam Moghadam

*Shahid Beheshti Medical University (M.C)
Tehran,
Iran*

Dr. Okeke Ikechukwu Linus

*Department of Surgery, University of Ibadan
Nigeria.*

Dr. Rajesh Kumar Patel

*Centre for Analysis and Learning in Livestock and Food
(CALF)
National Dairy Development Board (NDDB)
Anand- 388 001 (Gujarat)
INDIA*

Dr. Pooja Ralli-Jain

*Department of Pathology and Laboratory Medicine
University of California Irvine,
Irvine, California,
U.S.A.*

Dr. Meltem Sesli

*College of Tobacco Expertise,
Turkish Republic, Celal Bayar University 45210,
Akhisar, Manisa,
Turkey*

Dr. Reda H. Sammour

*Tanta University, Faculty of Science, Tanta,
Egypt*

Dr. Seyed Soheil Saeedi Saravi

*Mazandaran University of Medical sciences, Sari,
Iran*

Dr. R. Senthil Kumar

*St. Matthew's University, School of Medicine
Grand Cayman
Cayman Islands*

Dr. Mohammad Reza Shakibaie

*Kerman University of Medical Sciences, Kerman,
Iran*

Dr. Srividya Shivakumar

*Dept of Microbiology,
CPGS, Jain university,
Bangalore*

Dr. Shashideep Singhal

*The Brooklyn Hospital Center
NewYork-Presbyterian Healthcare System
Brooklyn,
NY.*

Dr. Sripada M. Udupa

*International Center for Agricultural Research in the
Dry Areas (ICARDA),
B.P. 6299, Rabat Instituts, Rabat,
Morocco.*

Dr. Wei Wu

*Institute for Biocomplexity and Informatics
Department of Bio Science
The University of Calgary
Canada*

Dr. Xiao-Bing Zhang

*Molecular Regeneration Laboratory, MC1528B
11234 Anderson Street
Loma Linda, CA 92350*

Prof. Dr. Ozfer Yesilada

*Inonu University
Faculty of Arts and Sciences
Department of Biology
44280 Malatya
Turkey*

Dr. Edson Boasquevisque

*Universidade do Estado do Rio de Janeiro- UERJ
Av 28 de setembro, 87, fundos (LMMC-IBRAG).
Vila Isabel, city: Rio de Janeiro/ RJ
Brasil*

Dr. Abhilash M.

*The Oxford College of Engineering
Hosur Road, Bangalore - 560068*

Dr. Nasar Uddin Ahmed

*Department of Genetics and Plant Breeding
Patuakhali Science and Technology University
Dumki, Patuakhali-8602
Bangladesh*

Dr. Mervat Morsy EL- Gendy

*Chemistry of Natural and Microbial Products
Department,
National Research Center, Dokki, Cairo,
Egypt*

Dr. Gjurmakch Aliev

*Health Science and Healthcare Administration
Program,
University of Atlanta, Atlanta, Georgia,
USA*

Dr. Muhammad Asgher

*Department of Chemistry and Biochemistry,
University of Agriculture,
Faisalabad,
Pakistan*

Dr. Anand Bharatkumar

*Parul Institute of Pharmacy, Limda, Waghodia,
Vadodara*

Dr. Chinmoy Kumar Bose,

*Netaji Subhash Chandra Bose Cancer Research
Institute
16A, Park Lane, Park Street, Kolkata 700 016,
India.*

Dr. Mousumi Debnath

*Jaipur Engineering College and Research Centre
(JECRC) Department of Biotechnology,
Shri Ram ki Nangal, Via Vatika ,Tonk Road , Jaipur-
303905 ,
India*

Dr. Dolan C. Saha

*Dept. of Biochemistry and Molecular Biology,
Faculty of Medicine,
University of Calgary,
Canada*

Dr. Ramasamy Harikrishnan

*Department of Aquatic Biomedical Sciences
School of Marine Biomedical Science
College of Ocean Sciences
Jeju National University
Jeju city, Jeju 690 756,
South Korea*

Dr. Abdul Haque

*Health
Biotechnology division, nibge,
Faisalabad,
Pakistan*

Dr. Kuvalekar Aniket Arun

*Interactive Research School for Health Affairs
(IRHSA),
Bharati Vidyapeeth University, Pune, Maharashtra,
India*

Dr. Asit Ranjan Ghosh

*School of Bio Science & Technology,
Division of Medical Biotechnology,
Vellore Institute of Technology (VIT) University,
Vellore-632014,
India*

Dr. Prasanna Kumar Santhekadur

*Department of Human and Molecular Genetics
Virginia Commonwealth University
Richmond,
VA*

Dr. Majid Sattari

*Rice Research Institute of Iran
Iran*

Dr. Mihael Cristin Ichim

*National Institute Research and Development for
Biological Sciences /
"Stejarul" Research Centre for Biological Sciences
Alexandru cel Bun St., 6, Piatra Neamt, 610004,
Romania*

Dr. Sailas Benjamin

*Enzyme Technology Laboratory
Biotechnology Division
Department of Botany
University of Calicut
Kerala - 673 635
India*

Dr. Sreeramanan Subramaniam

*School of Biological Sciences,
Universiti Sains Malaysia (USM),
Minden Heights, 11800, Penang,
Malaysia*

Dr. Vijai Kumar Gupta,

*Department of Biochemistry, NUI, Galway,
Ireland*

Dr. Vitor Engrácia Valenti

*Universidade Federal de São Paulo
Rua Napoleão de Barros, 715, Térreo
São Paulo, SP
Brazil.*

Dr. Ravindra Pogaku

*School of Engineering and IT
Universiti Malaysia Sabah
88999 Kota Kinabalu
Sabah,
Malaysia*

Dr. Ahmed Eid Abdel-Hamid Eweis Fazary

*School of Pharmacy,
College of Medicine,
National Taiwan University, Taipei 100,
Taiwan.*

Dr. Mohammad Hashemi

*Dept. of Clinical Biochemistry,
School of Medicine,
Zahedan University of Medical Sciences,
Zahedan,
Iran*

Dr. Hesham, Abd El-Latif

*Genetics Department,
Assiut University, Assiut 71516,
Egypt.*

Prof. Jia-ying Xin

*College of Food Engineering
Harbin University of Commerce
138 Tongda Road
Daoli District
Harbin 150076, Heilongjiang
P.R. China*

Dr. Kabir Mohammad Humayun

*Plant Molecular Biotech Lab
Department of Medical Biotechnology
College of Biomedical Science
Kangwon National University
Kangwon-do, Chuncheon, 200-701
South Korea*

Dr. Kalpesh Gaur

*Geetanjali College of Pharmaceutical Studies Manwa
Khera,
Udaipur- 313002. Rajasthan,
India*

Dr. Meganathan, Kannan

*Center for Biologics Evaluation and Research (CBER),
U.S. Food and Drug Administration (FDA),
Bldg. NIH 29A, Room 2C-10,
8800 Rockville Pike,
Bethesda, MD 20892.
USA.*

Assist. Prof. Ali Karadeniz

*Department of Physiology,
Faculty of Veterinary Medicine,
University of Atatürk 25240 ERZURUM
Turkey*

Dr. Matthew Kostek

*Department of Kinesiology
University of Connecticut
Storrs CT*

Dr. Tansu Kucuk

*Gulhane School of Medicine
Department of Obstetrics and Gynecology
Etlik 06018 Ankara,
Turkey*

Dr. Kuo-Sheng Hung

*Department of Neurosurgery
Taipei Medical University - Wan Fang Medical Center
111 Section 3, Hsing-Long Rd,
Taipei 116,
Taiwan*

Dr. V. Manju

*Department of Biochemistry,
Periyar University,
Salem -11.*

Dr. Mbagwu Ferdinand Nkem

*Department of Plant science and Biotechnology,
Faculty of Science,
Imo State University
Nigeria.*

Dr. Anand Pithadia

*Parul Institute of Pharmacy
Vadodara, Gujarat,
India*

Dr. Radhakrishnan Ramaraj

*Department of Internal Medicine
University of Arizona
Tucson 85724
AZ*

Dr. M. Rasool

*School of Bio Sciences and Technology,
VIT University,
Vellore-632104, Tamil Nadu,
India*

Dr. Reda A.I. Abou-Shanab

*Genetic Engineering & Biotechnology Research
Institute (GEBRI)
Mubarak City for Scientific Research and Technology
Applications
New Burg El-Arab City, Universities and Research
Institutes
Zone, P.O. 21934, Alexandria,
Egypt.*

Dr. MR. Pravin Babarao Suruse

*Department of Pharmaceutics
Sharad Pawar College of Pharmacy
Wanadongri, Hingna Road
Nagpur- 441 110. (M. S.)*

Dr. Jan Woraratanadharm

*GenPhar, Inc.,
Mount Pleasant,
SC*

Dr. Serap Yalin

*Mersin University Pharmacy Faculty
Department of Biochemistry, Mersin
Turkey*

Dr. YongYong Shi

*Bio-X Center,
Shanghai Jiao Tong University,
Hao Ran Building, 1954 Hua Shan Road,
Shanghai 200030,
PR China*

Dr. Jyotdeep Kaur

*Department of Biochemistry,
Post Graduate Institute of Medical Education and
Research (PGIMER),
Chandigarh*

Dr. Rajkumar

*Dept. Of Radiation Biosciences,
Institute of Nuclear Medicine and Allied Sciences
Brig. S.K. Mazumdar Road, Timarpur,
Delhi-110054
India*

Dr. Meera Sumanth

*Visveswarapura Institute of Pharmaceutical Sciences,
22nd Main, 24th Cross, B.S.K II stage,
Bangalore-560070
Karnataka,
India.*

Dr, Jai S. Ghosh

*Department of Microbiology,
Shivaji University,
Kolhapur 416004,
India*

Prof. Dr. Alaa H. Al-Charrakh

*Babylon University, College of Medicine.
Dept. of Microbiology
Hilla, Iraq*

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types

Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.

Decisions will be made as rapidly as possible, and the Journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AJMR to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length.. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by 'et al'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998;

1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001) References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. *Afr. J. Biotechnol.* 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant *Staphylococcus aureus* in community-acquired skin infections. *Emerg. Infect. Dis.* 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob. Agents Chemother.* 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). *Microbiology: Concepts and Applications*. McGraw-Hill Inc., New York, pp. 591-603.

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a \$650 handling fee. Publication of an article in the International Journal for Biotechnology and Molecular Biology Research is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances

Copyright: © 2013, Academic Journals.

All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Disclaimer of Warranties

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the IJBMBR, whether or not advised of the possibility of damage, and on any theory of liability.

This publication is provided "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.

Table of Contents: Volume 4 Number 6 October, 2013

ARTICLES

Research Articles

- Micropropagation of the Indian Birthwort *Arsitolochia indica* L** 86
Syed Naseer Shah, Amjad M. Husaini and Fatima Shirin
- A quick bud breaking response of a surface model for rapid clonal propagation in *Centella asiatica* (L.)L** 93
AK Bhandari, M Baunthiyal, VK Bisht, Narayan Singh and JS Negi

Full Length Research Paper

Micropropagation of the Indian Birthwort *Aristolochia indica* L.

Syed Naseer Shah¹, Amjad M. Husaini^{2*} and Fatima Shirin¹

¹Genetics and Plant Propagation Division, Tropical Forest Research Institute, Mandla Road, Jabalpur 482 021, India.

²Centre for Plant Biotechnology, Division of Biotechnology, SKUAST-K, Shalimar, Srinagar-191121, India.

Accepted 9 September, 2013

Aristolochia indica L. is a medicinal woody perennial climber plant of immense pharmaceutical value. The species is endangered with possible extinction due to its indiscriminate harvesting as raw material for pharmaceutical industry, to manufacture drugs against cholera, inflammation, biliousness, dry cough and snake bite. A rigorous attempt has been made for development of *in vitro* propagation procedure for this species, involving four steps, namely: culture establishment, shoot multiplication, rooting and hardening. Aseptic cultures were established by growing nodal segments (1 to 1.5 cm) as explants on Murashige and Skoog (MS) medium containing 5.0 µM N⁶-Benzyladenine (BA). Five nutrient media, MS, Woody Plant Medium (WPM), Gamborg Medium (B5), Nitsch and Nitsch Medium (NN), and Schenk and Hildebrandt Medium (SH) supplemented with different cytokinins and auxins at a concentration of 10.0 µM were used in this study. Ads at 10.0 µM proved optimum for *in vitro* shoot multiplication. The treatment resulted in 100% shoot number per explant at 15 days and 61.9% at 30 days on MS medium, 65.2% node number per shoot at 15 days and 196.2% at 30 days on WPM medium and 147.5 and 366.6% node number per explant at 30 days after inoculation on MS medium. The *in vitro* multiplied shoots were used for rooting experiment. Five nutrient media (MS, WPM, B₅, NN and SH) and three auxin sources 10.0 µM each (IBA, IAA and NAA). SH medium with 10.0 µM NAA induced 327.8% rooting at 21 days and 654.8% at 28 days and root number per explant 4300% at 21 and 394% at 28 day after inoculation. The *in vitro* propagated hardened plants exhibited excellent growth on transfer to natural condition.

Key words: *Aristolochia indica* L, *in vitro* propagation, N⁶-Benzyladenine.

INTRODUCTION

Aristolochia indica L. (family- Asclepiadaceae.) is a perennial climber with greenish whitish woody stem growing throughout India especially in the tropical and sub-tropical regions. The active constituent "Aristolochic acid" is potent drug used in Ayurvedic, Siddha and Homeopathy systems of medicines. Roots are widely used in joint pains and seeds in inflammation, biliousness, dry cough and dyspepsia. The juice of leaves or roots is said to be a specific antidote for cobra

poisoning (Kirtikar and Basu, 1987). The species is rare and endangered with extinction due to its indiscriminate collection and over exploitation from natural resources for commercial purpose by pharmaceutical industries (Rahman, 2001). The conventional propagation is hampered due to low seed viability and poor rooting of vegetative cuttings and emphasizes need for the alternative *in vitro* propagation method for large scale multiplication, improvement and conservation of the

*Corresponding author. E-mail: amjadhusaini@yahoo.com.

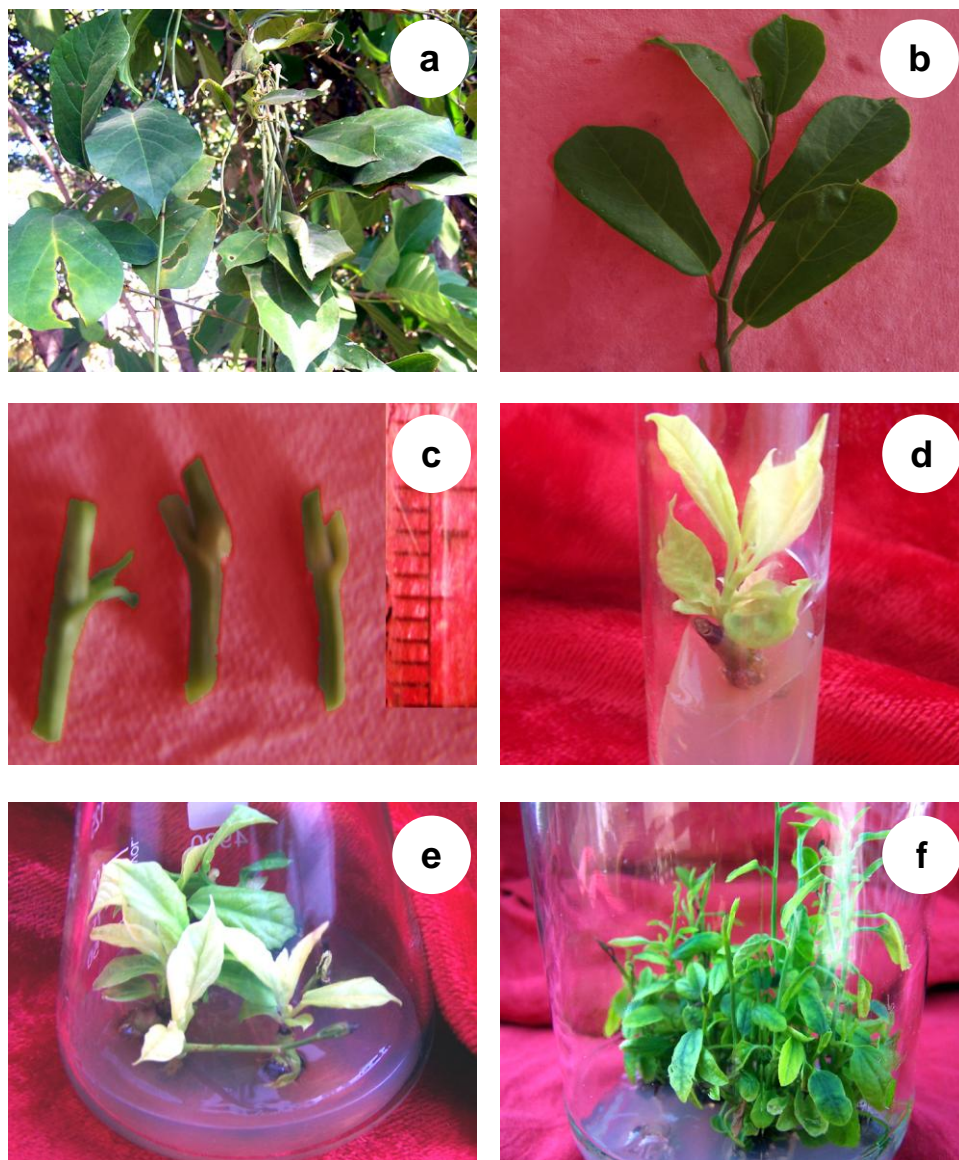


Figure 1. Explant collection, culture establishment and shoot multiplication in *Aristolochia indica* L.; (a) mother plant, (b) a twig, (c) nodal explants, (d) the *in vitro* culture establishment and (e-f) the *in vitro* shoot multiplication.

species. The objective of the study was to develop an efficient protocol for its micropropagation.

MATERIALS AND METHODS

The selected (mother) plant from Jabalpur area of Madhya Pradesh, India (Figure 1a) was used to collect twig (s) (Figure 1b), which were washed thoroughly for 15 min under running water for removing the surface debris. The washed twigs were defoliated and cut into nodal explants (approximately 1 to 1.5 cm long and 0.5 to 0.6 cm diameter) (Figure 1c). These explants were washed with 2% Cetrimide® and kept for 10 min with constant vigorous (shaking 150 rpm) on an orbital shaker incubator followed by rewashing 4 to 5 times with distilled water to remove traces of Cetrimide®. The washed explants were sterilized for 5 min with HgCl₂ (0.1%) and

Bavistin® (1.0%) in the laminar flow cabinet. Finally, the surface sterilized nodal explants were rinsed 4 to 5 times with sterile distilled water and inoculated on MS medium (Murashige and Skoog, 1962) supplemented with 5.0 µM BA for culture establishment (Figure 1d).

The *in vitro* shoot multiplication (Figure 1e-f) was standardized through a factorial randomized experiment, using single nodal segments from established cultures. In this experiment we screened five nutrient media [MS (Murashige and Skoog, 1962), WPM (Lloyd and McCown, 1980), B₅ (Gamborg et al., 1968), NN (Nitsch and Nitsch, 1969) and SH (Schenk and Hildebrandt, 1972)] along with 10.0 µM each of three cytokinins (BA, TDZ, Ads), and their combinations on shoot number per explant, node number per shoot and node number per explant at 15 and 30 days after inoculation. In second experiment, five nutrient media (MS, WPM, B₅, NN and SH) and three auxins (IBA, IAA and NAA) at

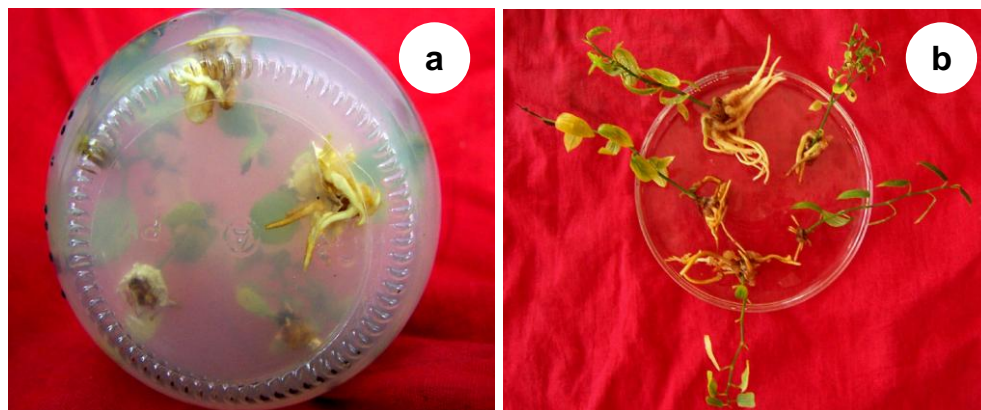


Figure 2. The *in vitro* induction of adventitious root in *Aristolochia indica* L. roots formation on semi-solid medium at (a) 21 and (b) 28 days after inoculation.

concentrations of 10.0 μM and their effect on rooting and root number was recorded at 21 and 28 days after inoculation (Figure 2).

Culture conditions

The inorganic salts used for preparation of culture medium were obtained from Qualigens Pvt. Ltd., India and phytohormones and B vitamins from Sigma Chemicals Pvt. Ltd., India. The medium contained 3% (w/v) sucrose, 0.8% (w/v) agar (Hi-Media chemical Ltd., India). The pH of the medium was adjusted to 6.0 before autoclaving for 15 min at 1.06 kg cm^{-2} (121°C). Explants were cultured in a 150 ml conical Borosil® flasks containing 40 ml semi-solid medium. For *in vitro* shoot multiplication and rooting experiment, the cultures were incubated at $25 \pm 2^\circ\text{C}$ under 16 h illuminations with fluorescent light (50 $\mu\text{mol Em}^{-2} \text{s}^{-1}$).

Hardening and transplantation

The *in vitro* raised plantlets were removed from rooting medium washed with distilled water and the plantlets were subsequently transferred to root trainers containing autoclaved soilrite (Figure 3a) and covered with perforated polythene to maintain humidity which were kept under culture room conditions for about 10 days. Subsequently, they were transferred to perforated polythene bags and kept initially in washing room for 5 days and finally transferred to natural condition (Figure 3b-d).

Statistical analysis

Each experiment had three replicates for *in vitro* shoot multiplication and rooting. Each replicate had 10 propagules. The data were subjected to two way (factor) analysis of variance for both the experiments with "F" test for ascertaining level of significance. If the data were found significant at $p \leq 0.05$, $\text{LSD}_{0.05}$ was computed for comparison of treatment means.

RESULTS

In vitro shoot multiplication

The effect of cultured media, cytokinin sources and their

all possible combinations on shoot number per explant, node number per shoot and node number per explant at both the stages of sampling was recorded.

Shoot number per explant

SH medium produced maximum shoot number per explant at 15 days and MS medium at 30 days (Table 1). The enhancement of shoot number per explant in SH medium was 100% in comparison to B_5 medium at 15 days and 61.90% in MS medium at 30 days after inoculation in comparison to that in B_5 medium. Further BA had significantly maximum shoot number explant⁻¹ at both stages and was statistically equalled by Ads at 15 days. Shoot number per explant in BA was 1333%, 76% more than that of TDZ at 15 and 30 days, respectively. MS medium with 10.0 μM BA produced maximum shoot numbers per explant which was 189% at 30 days after inoculation.

Node number per shoot

SH medium produced maximum node number per shoot at 15 days and WPM medium at 30 days (Table 2). The enhancement of node number per shoot in SH medium was 65.2% more than that obtained in NN medium at 15 days and 139% at 30 days in comparison to that in B_5 medium which produced the lowest value for the parameter at both stages of sampling. BA induced maximum node number per shoot, which was enhanced by 61% at 15 days and 239% at 30 days in comparison with TDZ. Maximum node number per shoot was observed on SH medium with Ads, at 15 days and WPM 10.0 μM Ads at 30 days. NN medium produced minimum effect on node number per shoot at both the stages of sampling at 15 days and B_5 medium at 30 days.

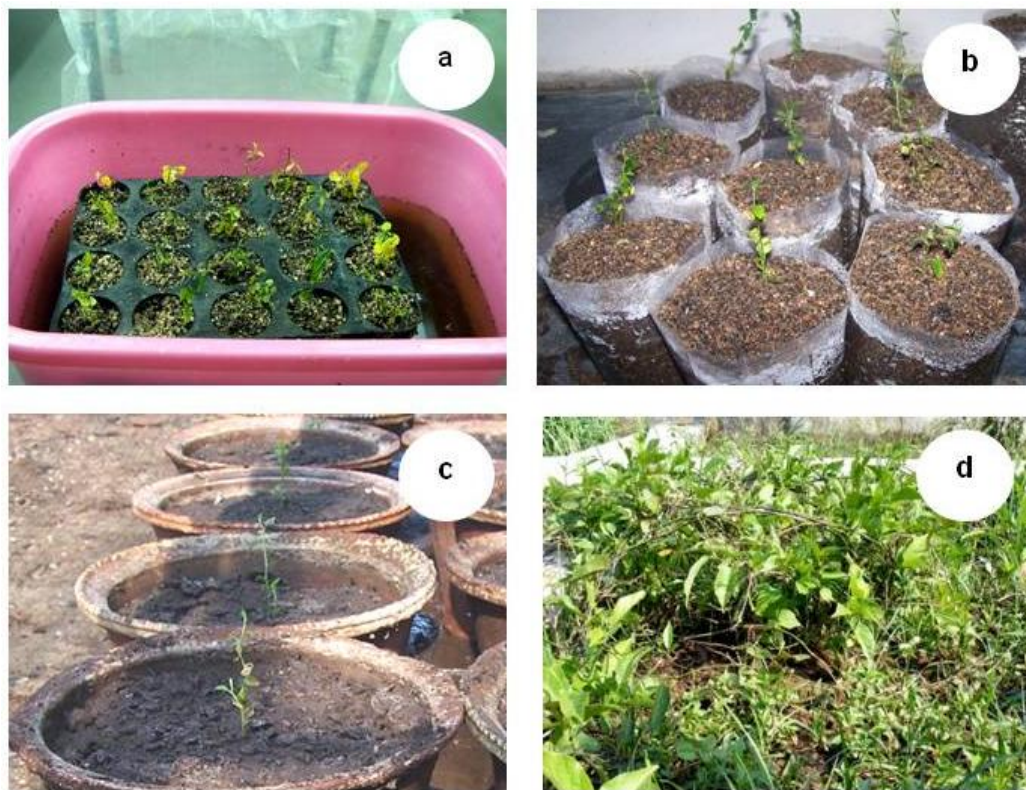


Figure 3. Hardening and acclimatization of the *in vitro* raised plantlets of *Aristolochia indica* L. Plantlets transferred to root trainers (a) and covered with polythene (b) placed in the culture room, (c) hardened plantlets transferred to polythene bags and (d) growth of the plantlets in the open environment.

Table 1. Effect of culture media and different cytokinins on shoot number per explant in *Aristolochia indica* L. at two stages of sampling.

Cytokinin source (C)	Culture media(M)											
	Inoculation days											
	15 Days					30 Days						
	MS	WPM	B5	NN	SH	Mean	MS	WPM	B5	NN	SH	Mean
BA	1.00	1.00	0.39	0.56	1.11	0.81	2.89	1.73	1.06	1.67	1.45	1.76
TDZ	0.33	0.00	0.00	0.00	0.00	0.06	1.00	1.00	1.00	1.00	1.00	1.00
Ads	0.78	1.00	0.72	0.72	1.11	0.86	1.17	1.17	1.11	1.78	1.45	1.33
Mean	0.70	0.66	0.37	0.42	0.74		1.70	1.30	1.05	1.48	1.30	

Variable	LSD _(0.05)	
	15Days	30Days
C	0.16	0.13
M	0.20	0.17
C *M	NS	0.29

Node number per explant

SH and MS medium induced maximum node number per explant at 15 days and at 30 days respectively (Table 3). The enhancement of node number per explant in SH

medium was 147.6% at 15 days and 366.6% in MS medium at 30 days as compared to B₅ medium. BA and Ads had significantly maximum node number per explants at 15 and 30 days, respectively. BA enhanced node number per explants by 48% at 15 days and Ads by

Table 2. Effect of culture media and different cytokinins on node number per shoot in *Aristolochia indica* L. at two stages of sampling.

Cytokinin sources (C)	Culture media(M)											
	Inoculation days											
	15 Days						30 Days					
	MS	WPM	B5	NN	SH	Mean	MS	WPM	B5	NN	SH	Mean
BA	1.28	1.55	1.17	1.28	2.78	1.61	4.04	2.67	1.11	2.28	2.72	2.57
TDZ	1.00	1.00	1.00	1.00	1.00	1.00	1.78	1.06	1.00	1.00	1.06	1.18
Ads	1.56	2.22	1.06	1.17	1.83	1.56	3.50	5.78	1.11	4.28	5.32	4.00
Mean	1.28	1.60	1.80	1.15	1.90		3.10	3.17	1.07	2.52	3.03	

Variable	LSD _(0.05)	
	15 Days	30 Days
C	0.19	0.47
M	0.25	0.61
C *M	0.43	1.06

Table 3. Effect of culture media and different cytokinins on node number per explant in *Aristolochia indica* L. at two stages of sampling.

Cytokinin sources (C)	Culture media(M)											
	Inoculation days											
	15 Days						30 Days					
	MS	WPM	B5	NN	SH	Mean	MS	WPM	B5	NN	SH	Mean
BA	1.28	1.56	0.81	0.67	3.07	1.48	11.72	4.33	1.36	3.97	4.27	5.13
TDZ	1.00	1.00	1.00	1.00	1.00	1.00	1.06	1.00	1.00	1.00	1.06	1.02
Ads	1.22	2.22	0.76	0.81	2.03	1.41	4.00	6.80	1.27	7.55	7.73	5.47
Mean	1.16	1.60	0.86	0.82	2.03		5.60	4.04	1.20	4.18	4.35	

Variable	LSD _(0.05)	
	15 Days	30 Days
C	0.21	0.85
M	0.28	1.10
C *M	0.48	1.91

436% at 30 days in comparison to TDZ, which produced the lowest value for the parameter. As for interaction, SH medium with 10.0 μ M Ads registered the highest value for the parameter at 30 days after sampling.

***In vitro* adventitious rooting**

Auxin sources and their combinations with different media induced significant rooting and root number per explant at both the stages of sampling.

Percent rooting

SH medium produced significantly high percent of rooting. The enhancement of rooting in SH medium was 327.8% at 21 days and 655% at 28 days in comparison

to MS medium. MS, B₅, NN and WPM produced minimum effect on rooting. NAA produced significantly maximum rooting (%), which was 228 at 21 days and 443.7% at 28 days after inoculation in comparison to IAA producing minimum value for rooting. SH medium with 10.0 μ M NAA maximum rooting at both stages of sampling (Table 4).

Root number per explants

SH medium produced maximum root number per explant at both the stages of sampling. The enhancement of root number per explant was 4300% at 21 days and 394% at 28 days after inoculation in comparison with WPM, MS and NN medium. NAA was found to have significant effect on root number per explant at both stages of sampling and resulted in 800% at 21 days and 2900% at 28 days more than that obtained in IAA. SH medium

Table 4. Effect of culture media and different auxins on percent of rooting in *Aristolochia indica* L. at two stages of sampling.

Auxin sources (A)	Culture media (M)											
	Inoculation days											
	Rooting at % 21 Days						Rooting at % 28 Days					
	MS	WPM	B5	NN	SH	Mean	MS	WPM	B5	NN	SH	Mean
IBA	0 (4.16)	0 (4.16)	0 (4.16)	16.66 (24.06)	0 (4.16)	3.33 (8.14)	0 (4.16)	0 (4.16)	5.55 (10.79)	16.66 (24.06)	0 (4.16)	4.44 (9.47)
IAA	0 (4.16)	0 (4.16)	0 (4.16)	0 (4.16)	0 (4.16)	0 (4.16)	0 (4.16)	0 (4.16)	0 (4.16)	5.55 (10.79)	0 (4.16)	1.11 (5.49)
NAA	5.55 (10.79)	0 (4.16)	0 (4.16)	0 (4.16)	50 (45)	11.11 (13.65)	22.22 (24.4)	0 (4.16)	16.66 (24.06)	5.55 (10.79)	100 (85.84)	28.89 (29.85)
Mean	1.9 (6.4)	0 (4.16)	0 (4.16)	5.6 (10.8)	16.7 (17.8)		7.4 (10.9)	0 (4.16)	7.4 (13.00)	9.3 (15.2)	33.3 (31.4)	
Variable	LSD (0.05)											
	21 Days	28 Days										
A	2.21	5.49										
M	2.85	7.08										
A*M	4.94	12.27										

Table 5. Effect of culture media and different auxins on root number per explant in *Aristolochia indica* L. at two stages of sampling.

Auxin sources (A)	Culture media(M)											
	Inoculation days											
	Root number 21 Days						Root number 28 Days					
	MS	WPM	B5	NN	SH	Mean	MS	WPM	B5	NN	SH	Mean
IBA	0.00	0.00	0.00	0.16	0.00	0.03	0.00	0.00	0.28	0.39	0.00	0.13
IAA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.03
NAA	0.05	0.00	0.00	0.00	1.33	0.27	0.66	0.00	0.44	0.11	3.28	0.90
Mean	0.01	0.00	0.00	0.05	0.44		0.22	0.00	0.24	0.22	1.01	
Variable	LSD (0.05)											
	21 Days	28 Days										
A	0.16	0.27										
M	0.20	0.35										
A*M	0.36	0.61										

along with 10.0 μ M NAA was found to have significant effect on root number per explant at 21 and 28 days after inoculation (Table 5).

DISCUSSION

The micro-propagation of *A. indica* comprises four steps, namely: establishment of culture from nodal explants, shoot multiplication, root induction and hardening and

acclimatization. The present investigation was intended for the standardization of culture medium and plant growth regulators at second and third steps followed by hardening procedure. For shoot multiplications, the best *in vitro* combination was SH medium supplemented with 10.0 μ M Ads. There is no published report on the *in vitro* shoot multiplication of *A. indica* using SH medium. The suitability of SH medium in the present study contrasts with earlier reports of micropropagation for this species wherein MS medium was found to be the most effective

(Siddique et al., 2006a; 2006b; Pattar and Jayraj, 2012). The results indicate that the species requires low amount of nitrogen for growth and differentiation of new shoots. Adenine sulphate was found as the most suitable cytokinin for shoot multiplication. Similar results have been reported in the medicinal plant *Cichorium intybus* also, where multiple shoots proliferation was observed on medium supplemented with BA, IAA and adenine sulphate (Nadagopal and Ranjitha Kumari, 2006).

For *in vitro* rooting also, better performance was obtained on SH medium. High concentration of thiamine (Vitamin B₁) included in SH medium seems to be synergistic with auxins for facilitation of rhizogenesis as reported in teak by Ansari et al. (2002). Of the various auxin treatments, NAA was found to be the best auxin for *A. indica*. Superiority of NAA for *in vitro* rooting may be attributed to its synthetic nature and stability. Further, NAA also eludes the auxin oxidizing/ degrading enzyme systems of the plants (Jacobs, 1972). IAA was found to be inferior to both NAA and IBA. In literature also there are reports of IBA and NAA being more effective than IAA, because of the instability of the latter (Gaspar and Coumans, 1987).

Conclusion

The study demonstrates successful development of *in vitro* propagation procedure for *A. indica*. The procedure offers a potential system for conservation and mass propagation using explants derived from mature plants. SH (medium supplemented with 10.0 µM Ads has been found the best for efficient and rapid multiplication of *in vitro* shoots, while SH medium supplemented with 10.0 µM NAA for optimum induction of *in vitro* adventitious roots. Further, the hardening procedure reported here ensures 70 to 80% field survival of micropropagated plants of *A. indica*.

REFERENCES

- Ansari SA, Sharma S, Pant NC, Mandal AK (2002). Synergism between IBA and thiamines for induction and growth of adventitious roots in *Tectona grandis*. J Sustain. For. 15:99-112.
- Gamborg OL, Miller RA, Ojima K (1968). Nutrient requirements of suspension cultures of soya bean root cells, Exp. Cell Res. 50:151-58.
- Gaspar T, Coumans M (1987). Root formation *In*: J.M. Bonga and D.J. Durzan (eds). Cell and Tissue Culture in Forestry, Specific Principles and Methods: Growth and Developments, Martinus Nihoff Publishers, Dordrecht, 2:202-217.
- Jacobs WP (1972). The movement of plant hormones: auxins, gibberellins and cytokinins". In D.J. Carr (ed.), Plant Growth Substances, Springer, New York. pp. 701-709.
- Kirtikar KR, Basu LM (1987). Indian Med. Plants. pp. 2117-2118.
- Lloyd G, McCown B (1980) Commercially feasible micropropagation of mountain laurel *Kalmia latifolia* by use of shoot tip culture, Comb. Proc. Int. Plant prop. Soc. 30:421-27.
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiol. Plant. 15:473-97.
- Nadagopal S, Ranjitha Kumari BD (2006). Adenine sulphate induced high frequency shoot organogenesis in callus and *in vitro* flowering of *Cichoriumintybus* L. cv. Focus - a potent medicinal plant, Acta Agric. Slov. 87(2):415-425.
- Nitsch JP, Nitsch C (1969). Haploid plants from pollen grains, Sci. 163:85-87.
- Pattar PV, Jayraj M (2012). *In vitro* Regeneration of Plantlets from Leaf and Nodal explants of *Aristolochia indica* L. - An important threatened medicinal plant. Asian Pac. J. Trop. Biomed. 2(2):488-493.
- Rahman M (2001). Red data Book of Vascular plants. Bangladesh National Herbarium. Dhaka, Bangladesh.
- Schenk RU, Hildebrandt AC (1972). Medium and technique for induction and growth of monocotyledonous and dicotyledonous plant cell culture, Can. J. Bot. 50:199-204.
- Siddique NA, Bari MA, Pervin MM, Nahar N, Banu LA, Paul KK, Kabir MH, Huda AKMN, Ferdous KMK, Hossain MJ (2006a). Plant Regeneration from Axillary Shoots Derived Callus in *Aristolochia indica* Linn. an Endangered Medicinal Plant in Bangladesh, Pak. J Biol. Sci. 9:1320-323.
- Siddique NA, Kabir MH, Bari MA (2006b). Comparative *in vitro* study of plant regeneration from nodal segments derived callus in *Aristolochia indica* Linn. and *Hemidesmus indicus* (L.) R. Br. endangered medicinal plants in Bangladesh, J. Plant Sci. 1(2):106-118.

Full Length Research Paper

A quick bud breaking response of a surface model for rapid clonal propagation in *Centella asiatica* (L.)

AK Bhandari^{1*}, M Baunthiyal², VK Bisht¹, Narayan Singh¹ and JS Negi¹

¹Herbal Research and Development Institute (HRDI) - Mandal, Gopeshwar, Chamoli, Uttarakhand, India.

²Department of Biotechnology, G. B. Pant Engineering College- Ghurdauri, Pauri-Garhwal, Uttarakhand, India.

Accepted 9 September, 2013

Present investigation was planned to evaluate time period of bud breaking in *Centella asiatica* with different concentration of plant growth regulators, a medicinal herb distributed throughout the worldwide. For the study, concentrations were designed for response surface model describing bud breaking growth in optimum conditions. A combination of BAP (2 mg/L) + gibberellic acid (GA₃, 0.5 mg/L) was achieved at a best initial bud breaking at 8th hour. Longest time period taken for bud breaking was shown in combination of BAP (0.5 mg/L) + naphthalene acetic acid (NAA, 0.5 g / L) and BAP (0.1 mg/L) + adenine sulphate (0.5 mg/L) which was recorded at 84th hour. Half strength MS media was supplemented with IBA alone (2 mg/L) and in combination with IAA (0.5 mg/L) to attain an early *in vitro* rooting. Their interactions observed were statistically significant (P < 0.05).

Key words: *Centella asiatica*, bud, plant growth regulator, medicinal plant.

INTRODUCTION

Centella asiatica (L.) Urban, synonym *Hydrocotyle asiatica* (Family: Apiaceae) is a small perennial herb, commonly known as Mandukparni. In India, this species is mostly found in the swampy areas up to an altitude of 600 to 1800 m asl (Patra et al., 1998). Medicinally, *C. asiatica* used as memory enhancer and in the treatment of chronic diseases, mental disorders and neuropharmacological disorders like insomnia, insanity, depression, psychosis, epilepsy and stress (Chopra et al., 1980). The major bio-active ingredients in the plant are the triterpenes, asiatic acid, madecassic acid and their glycosides such as asiaticoside and madecassoside (Zheng and Qin, 2007). Due to the presence of these active ingredients, it possesses antileprotic, antifilarial, antibacterial, antifeedant, adaptogenic and antiviral

properties (Warrier et al., 1994). The roots contain many polyacetylenic compounds, the major compound being 8-acetoxyfalcariol (Loc and Tam, 2010).

Over-exploitation of *C. asiatica* from natural habitats for medicinal purposes causes depletion of plant population. There has been an increase interest in *in-vitro* culture techniques for mass multiplication of important species to overcome the pressure of over-exploitation and to restore species diversity (Patra et al., 1998; Tiwari et al., 2000; Bhandari et al., 2010). However, till date *in-vitro* technique has been applied only for < 20% of medicinally important species (Shukla et al., 1999). *In-vitro* propagation of *C. asiatica* was also carried out through leaf explants (Banerjee et al., 1999), axillary buds (George et al., 2004), stolons (Sampath et al., 2001), shoot tips

*Corresponding author. E-mail: arvinbhandari2001@yahoo.co.in.

(Sangeetha et al., 2003), callus cultures (Patra et al., 1998; Rao et al., 1999) and somatic embryogenesis (Martin, 2004). Besides all, there is information available on the methods to initiate early bud breaking, shoot formation and root initiation. Therefore, present study was designed to understand the effect of different PGRs in alone and in combination for bud breaking, shoot formation and root initiation. Present study will be useful in producing quality planting material with in short duration.

MATERIALS AND METHODS

Ex-plant selection

For the Rapid clonal propagation of *C. asiatica*, explants were collected from the Herbal garden (1545 m asl) of Herbal Research and Development Institute, Mandal, Gopeshwar (Chamoli) Uttarakhand, India; It is bounded by North Latitude 30° 27' 13.40" and East Longitude 79° 16' 21.61".

The media for clonal propagation was prepared by following Murashige and Skoog (1962), All Chemicals used for the research purpose were purchased from HiMedia Laboratories (Mumbai, India), and growth regulators were purchased from Sigma Chemical Co. (St Louis, MO) and HiMedia Laboratories, India. Cultures were established for the bud explants on MS medium (Murashige and Skoog, 1962) containing 58 mM sucrose and gelled with 0.7% (w/v) agar. The pH of medium was adjusted between 5.6 to 5.8 using 0.1 N HCL or 0.1 N NaOH solution prior to the autoclaving at 121°C and a pressure of 15 psi for 20 min then allowed to cool at room temperature. The explants thoroughly washed with running tap water for 15 to 20 min, then treated with 1% (v/v), Tween 20 solution and subsequently for 15 min with a sodium hypochlorite solution (0.5% active chlorine) in laminar air flow cabinet and finally the explants were washed thoroughly with autoclaved distilled water for several times to remove the traces of sodium hypochlorite. In support of surface disinfection, bud segments were trimmed from the cut ends in appropriate size, and cultured.

Culture conditions and *in-vitro* establishment of plantlets

For establishment of cultures, the surface disinfected explants were inoculated on full strength MS (Murashige and Skoog, 1962) basal medium having 3% of sucrose, semi-solidified with 0.7% (w/v) agar and supplemented with different concentrations of plant growth regulator viz. BAP (0.1 to 2 mg/L), adenine sulphate (0.1 to 0.5 mg/L) and gibberellic acid (0.5 mg/L). Half strength of MS medium supplemented with growth regulator IBA (0.5 to 2.0 mg/L) and NAA 0.1 to 0.5 mg/L was attempt for rooting. Each hormonal combination was tried in three replicates. 250 ml (Borosil, India) Conical flasks containing 20 ml of medium were used. Cultures vessels were used for incubated at 25 ± 1°C under a 16/8 h light/dark photoperiod with light provided by cool-white fluorescent lamps (Philips India, Mumbai, India) at a light intensity of 1000 lux. The multiplied cultures were taken out; every single shoot was excised and kept in small plastic cup filled with a mixture of soil: sand (1:1) for *ex-vitro* rooting.

Multiple shoots from bud induction

The explants were inoculated in semi-solid MS medium with

concentrations (0.1, 0.2, 1.5 and 2 mg/L) of BAP in alone, with combination of BAP (0.5 and 2 mg/L) along with gibberellic acid in 0.5 mg/L, BAP (0.1, 0.5, 1 and 2 mg/L) with adenine sulphate 0.5 mg/L and BAP (0.5 to 2 mg/L) with the combination of NAA (0.5 mg/L). Sub culturing was carried out at periodic intervals of three weeks.

Rooting of microshoots

Developed shoots having one or two nodes were excised and transferred to root induction medium comprising of ½ strength MS medium with 3% sucrose and supplemented with different concentrations of IBA (0.5, 1, 1.5 and 2.0 mg/L) in alone and (0.1, 0.2, 0.5, 1.5 and 2.0 mg/L) with IAA (0.5 mg/L) in combination. Number of roots per shoot and root length was score in alternate day.

Hardening and acclimatization

For *in vitro* hardening, rooted shoots were transferred to ¼ MS strength medium having 3% sucrose devoid of PGR for seven days in flasks. Thereafter, they were transferred to polybags containing a mixture of soil: sand: FYM manure (1:1:1) and kept for two weeks in mist-chamber under controlled condition (temp-25°C ± 2°C), humidity (65% ± 5%). Acclimatized plants were later shifted to soil in pots in agronet-shade house for one week and after that in field.

Statistical analysis

The data collected was subjected to the analysis of variance (ANOVA); using MS Excel 2007 for calculating the significance among different treatments and time of bud breaking and time of root initiation values at P < 0.05 were computed to compare means from various treatments.

RESULTS AND DISCUSSION

Initial study on *C. asiatica* was undertaken by Patra et al. (1998), Banerjee et al. (1999) and Tiwari et al. (2000). Tiwari et al. (2000) reported that initiation of nodal culture is better using different combination of plant growth regulators. The results of the present study on bud initiation, bud establishment and root initiation in MS medium supplemented with various combinations of growth regulators are presented in Tables 1 to 3. The earliest bud breaking in this study was achieved in BAP (2 mg/L) + GA₃ (0.5 mg/L). Initiation of bud breaking within 8 h of *in-vitro* culture in *C. asiatica* was reported first time in present study. Achieving early bud breaking is of importance as it produces quality planting material vis a vis reduces time and efforts. Different combinations of PGRs have also been reported to initiate bud breaking (Sen and Sharma, 1991). The longest time period (84 h) taken for bud breaking in present study was noticed in combination of BAP (0.5 mg/L) + NAA (0.5 mg/L) and BAP (0.1mg/L) + adenine (0.5 mg/L) which are presented in Tables 2 and 3.

BAP alone at higher concentration (2 mg/L) seems to initiate early bud breaking. Similar observations in

Table 1. Morphogenetic response of *C. asiatica* buds cultured on MS medium supplemented with different concentrations of BAP and Gibberellic acid (Bud breaking is found positively significant LSD = 24.25 (P < 0.05).

BAP (mg/L)	Gibberellic acid (mg/L)	Time of bud breaking (h)
Control	-	0
0.1		72
0.1		60
0.1		66
0.2		60
0.2		72
0.2		72
0.5	0.5	36
0.5	0.5	38
0.5	0.5	40
1.5		40
1.5		46
1.5		42
2	0.5	8
2	0.5	12
2	0.5	14
2		24
2		18
2		16

t-value= 2.02, LSD = 24.25 (P < 0.05)

Table 2. Morphogenetic response of *C. asiatica* buds cultured on MS medium supplemented with different concentrations of BAP and Adinine (Bud breaking is found positively significant LSD=19.36; P < 0.05).

BAP (mg/L)	Adinine sulphate (mg/L)	Time of bud breaking (h)
Control	-	0
0.1	0.5	84
0.1	0.5	80
0.1	0.5	78
0.5	0.5	72
0.5	0.5	68
0.5	0.5	66
1	0.5	48
1	0.5	44
1	0.5	40
2	0.5	24
2	0.5	20
2	0.5	18

t-value = 2.13, LSD =19.36; (P < 0.05).

Table 3. Morphogenetic response of *C. asiatica* buds cultured on MS medium supplemented with different concentrations of BAP and NAA (Bud breaking is found positively significant LSD=19.36; P < 0.05).

BAP (mg/L)	NAA (mg/L)	Time of bud breaking (h)
Control	-	0
0.5	0.5	84
0.5	0.5	80
0.5	0.5	76
2	0.5	48
2	0.5	46
2	0.5	42

t-value= 2.03, LSD=19.36; P < 0.05

Ocimum basilicum was found by Pattnaik and Chand (1996). In addition, BAP was found more efficient over kinetine (Kn) in *in-vitro* shoot proliferation in different species (Purohit, 1994; Martin, 2003). In *Swertia chirata*, BAP with higher concentration have optimal response for shoot proliferation (Chaudhuri, 2007; Pant et al., 2010). In present study, BAP in combination with GA₃ (with different concentrations) significantly (P < 0.05) enhance the rate of bud breaking, shoot proliferation and root initiation (Table 1, Figure 1A-E). Sharma and Sharma (2010) attributed this to the stimulating effects of various hydrolytic enzymes activities thus increasing availability of nutrients for growth. The result of the effects of BAP and GA₃ on shoot proliferation in present study was found comparable to the earlier reports. However, Tiwari et al. (2013) reported improved bud breaking using high concentration of BAP (5 mg/L) and improved root formation in combinations of BAP (4.0 mg/L) and IBA (0.5 mg/L). Karthikeyan et al. (2009) described the rapid clonal propagation through auxiliary shoot proliferation in *C. asiatica*. The shoot elongation with the treatment of BAP and GA₃ might be due to cell enlargement and increase in normal cell division (Karivartharaju and Ramakrishnan, 1985). Earliest root initiation was achieved alone in IBA (2 mg/L) and in combination of IBA (2 mg/L) and IAA (0.5 mg/L) (Tables 4 and 5).

Thus, it is concluded that *in-vitro* micro-propagation offer rapid clonal multiplication of elite clones and further helps in dissemination fulfilling the need of vis a vis to quality planting material. BAP (2 mg/L) in combination with GA₃ (0.5 mg/L) is recommended for effective and earliest bud breaking. Likewise, IBA (2 mg/L) is recommended for earliest rooting in *C. asiatica*.

ACKNOWLEDGEMENTS

The authors are grateful to technical staff Megha Sati and Shweta Semwal for assistance in carrying out the research

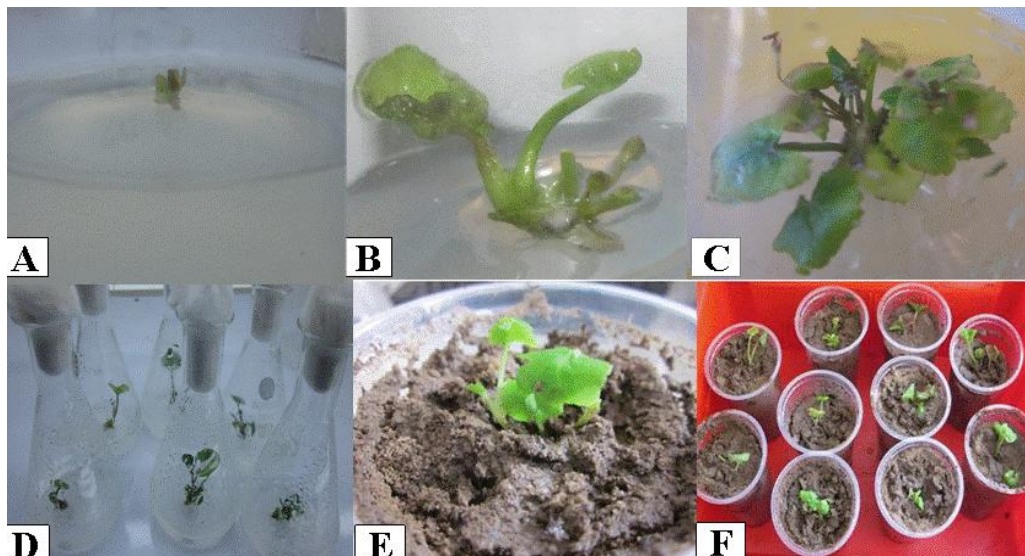


Figure 1. In vitro regeneration of *Centella asiatica*, via bud explants; (A) axillary bud induction on nodal segment MS medium+BAP (2 mg/L)+gibberellic acid (0.5 mg/L), (B) bud induction on nodal segment, (C) culture establishment, (D) multiplication of shoots in BAP (0.5 mg/L)+NAA 0.1 mg/L, (E) rooted plantlet containing a mixture of soil : sand : manure (1:1:1), (F) plantlet in soil for hardening containing a mixture of soil : sand : manure (1:1:1).

Table 4. Morphogenetic response of root initiation explants of *C. asiatica* cultured on half strength MS medium supplemented with different concentrations of IBA (Root initiation is found positively significant LSD=3.16; (P < 0.05).

IBA (mg/L)	Time of root initiation (Days)
Control	0
0.5	16
0.5	14
0.5	15
1	15
1	14
1	13
1.5	12
1.5	11
1.5	11
2	10
2	9
2	8

t-value= 2.07, LSD=3.16; (P < 0.05)

Table 5. Morphogenetic response of root initiation explants of *C. asiatica* cultured on half strength MS medium supplemented with different concentrations of IBA and IAA (Bud breaking is found positively significant LSD=3.12; (P < 0.05).

IBA (mg/L)	IAA (mg/L)	Time of root initiation (Days)
Control	0	0
0.1	0.5	18
0.1	0.5	16
0.1	0.5	16
0.2	0.5	18
0.2	0.5	16
0.2	0.5	16
0.5	0.5	15
0.5	0.5	14
0.5	0.5	13
1.5	0.5	12
1.5	0.5	11
1.5	0.5	10
2	0.5	10
2	0.5	9
2	0.5	9

t-value= 2.02, LSD=3.12; (P < 0.05)

work and Agriculture and Processed Food Products Export Development Authority, Ministry of Commerce and Industry, Government of India to provide financial support for instrument facility to the Institute under Herbal Analytical Laboratory Project (Grant no. FLR/059/2006-07/13692).

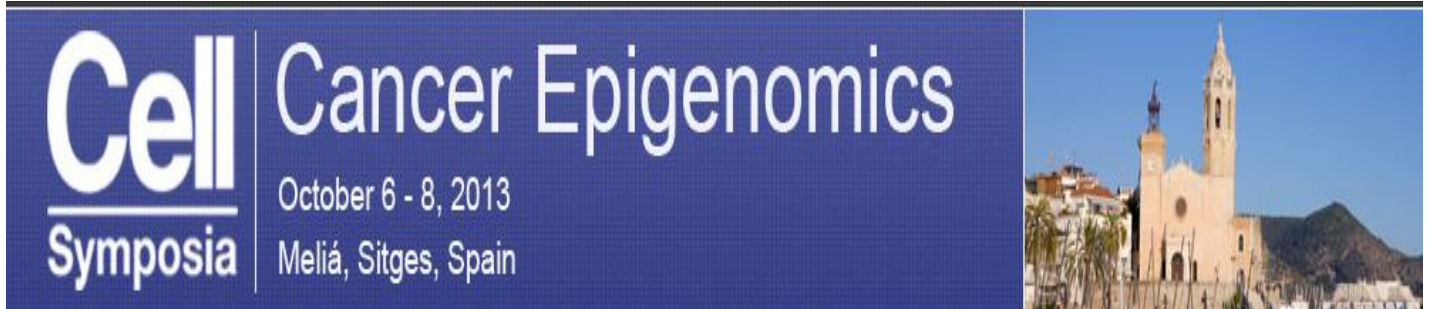
REFERENCES

Banerjee S, Zehra M, Kumar S (1999). *In vitro* multiplication of *Centella asiatica*, a medicinal herb from leaf explants. *Curr. Sci.* 76:147-148.

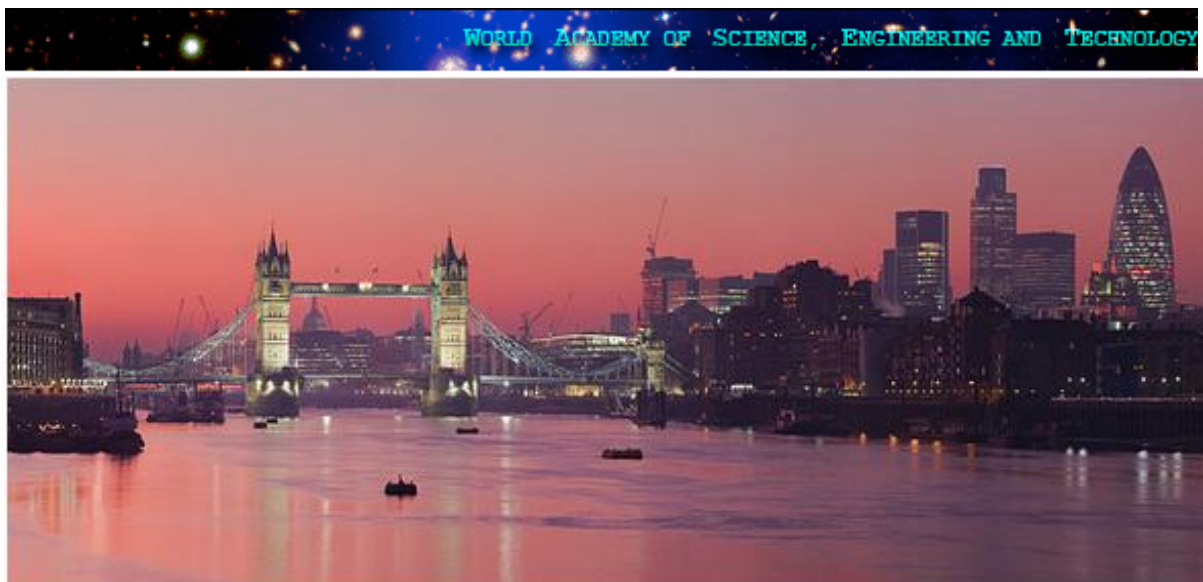
- Bhandari AK, Negi JS, Bisht VK, Bharti MK (2010). *In vitro* culture of *Aloe vera* - A plant with medicinal property. *Nat. and Sci.* 8(8):174-176.
- Chaudhuri RK (2007). Production of genetically uniform plants from nodal explants of *Swertia chirata* Buch.-Ham.ex Wall.: an endangered medicinal herb. *In-vitro Cell. Div. Biol. Plant* 43:467-472.
- Chopra RN, Nayar SL, Chopra IC (1980). *Glossary of Indian Medicinal Plants (Including the Supplement)*. CSIR, New Delhi.
- George S, Remashree AB, Sebastian D, Hariharan M (2004). Micropropagation of *Centella asiatica* L. through axillary bud multiplication. *Phytomorphology* 54:31-34.
- Karivartharaju TV, Ramakrishnan V (1985) Seed hardening studies in two varieties of ragi. *Indian J. Pl. Physiol.* 28:243-248.
- Karthikeyan K, Chandran C, Kulothungan S (2009). Rapid clonal multiplication through *in-vitro* axillary shoot proliferation of *Centella asiatica* L. *Ind. J Biotechnol.* 8:232-235.
- Loc NH, Tam NT (2010). An Asiaticoside production from *Centella (Centella asiatica* L. Urban) cell culture. *Biotech. Bioproc. Eng.* 15:1065-1070.
- Martin KP (2003). Rapid *in-vitro* multiplication and ex-vitro rooting of *Rotula aquatic* Lour., a rare rheophytic woody medicinal plant. *Pl. Cell Rep.* 21:415-420.
- Martin KP (2004). Plant regeneration through somatic embryogenesis in medicinally important *Centella asiatica* L. *In vitro Cell. Dev. Biol. Plant.* 40:586-591.
- Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays for tobacco tissue cultures. *Physiol. Plant.* 15:473-97.
- Pant M, Bisht P, Gusain PM (2010). *In vitro* propagation through axillary bud culture of *Swertia chirata* Buch.-Ham ex Wall: an endangered medicinal herb. *Int. J. Int. Bio.*10:48-53.
- Patra A, Rai B, Rout GR, Das P (1998). Successful plant regeneration from callus cultures of *Centella asiatica*. *Pl. Growth Regul.* 24:13-16.
- Pattnaik SK, Chand PK (1996). *In vitro* propagation of the medicinal herbs *Ocimum americanus* Pl. *Cell Reports* 15:846-850.
- Purohit SD (1994). Micropropagation of safed musli (*Chlorophytum borivilianum*), a rare medicinal herb. *Plant Cell Tiss. Org. Cult.* 39:93-96.
- Rao KP, Rao SS, Sadanandam M (1999). Tissue culture studies of *Centella asiatica*. *Indian J. Pharm. Sci.* 61:392-394.
- Sampath P, Muthuraman G, Jayaraman P (2001). Tissue culture studies in *Bacopa monnieri* and *Centella asiatica*. National Research Seminar on Herbal Conservation, Cultivation, Marketing and Utilization with Special Emphasis on Chattisgarh, The Herbal State, Raipur, Chattisgarh, India. 12:18-21.
- Sangeetha N, Buragohain AK (2003). *In vitro* method for propagation of *Centella asiatica* (L.) urban by shoot tip culture. *J. Plant Biochem. Biotechnol.* 12:167-169.
- Sen J, Sharma AK (1991). Micropropagation of *Withania somnifera* from germinating seeds and shoot tips. *Plant Cell Tiss. Org. Cult.* 26:71-73.
- Sharma S, Sharma RK (2010). Seed physiological aspects of pushkarmool (*Inula racemosa*), a threatened medicinal herb: response to storage, cold stratification, light and gibberellic acid. *Curr. Sci.* 99(12):1801-1806.
- Shukla A, Rasik AM, Jain GK, Shankar R, Kulshrestha DK, Dhawan BN (1999). *In vitro* and *in vivo* wound healing activity of asiaticoside isolated from *Centella asiatica*. *J. Ethnopharmacol.* 65:1-11.
- Tiwari C, Bakshi M, Vichitra A (2013). A rapid two step protocol of *in vitro* propagation of an important medicinal herb *Centella asiatica* Linn. *Afr. J. Biot.*12:1084-1090.
- Tiwari KN, Sharma NC, Tiwari V, Singh BD (2000). Micropropagation of *Centella asiatica* (L.) a valuable medicinal herb. *Plant Cell Tiss. Organ Cult.* 63:179-185.
- Warrier PK, Nambiar VP, Ramankutty C (1994). *Indian medicinal plants. A compendium of 500 species.* Vol-I, Orient Lonhman Pvt Ltd, Chennai, India: pp.52-55.
- Zheng CJ, Qin LP (2007). Chemical components of *Centella asiatica* and their bioactivities. *J. Chin. Integ. Med.* 5:348-351.

UPCOMING CONFERENCES

Cell Symposia: Cancer Epigenomics, Meliá, Spain, 6 Oct 2013



**International Conference on Biotechnology and Environment Engineering,
London, UK, 19 Jan 2014**



ICBEE 2014 : International Conference on Biotechnology and Environment Engineering

**GB, London
January 20-21, 2014**

Conferences and Advert

October 2013

Cell Symposia: Cancer Epigenomics, Meliá, Spain, 6 Oct 2013

3rd Journal Conference on Bioscience, Biochemistry and Bioinformatics, Paris, France, 12 Oct 2013

4th International Conference on Stem Cells and Cancer (ICSCC-2013): Proliferation, Differentiation and Apoptosis, Mumbai, India, 19 Oct 2013

13th Congress of the Asia-Pacific Federation for Clinical Biochemistry and Laboratory Medicine (APCCB 2013), Bali, Indonesia, 27 Oct 2013

11th Congress on Euro Fed Lipid, Antalya, Turkey, 27 Oct 2013

November 2013

2nd Conference of Cereal Biotechnology and Breeding (CBB2), Budapest, Hungary, 5 Nov 2013

January 2014

International Conference on Biotechnology and Environment Engineering, London, UK, 19 Jan 2014

International Journal for Biotechnology and Molecular Biology Research

Volume 4 Number 6 October 2013

ISSN 2141-2154



*Academic
Journals*