ABOUT IJPPB

The International Journal of Plant Physiology and Biochemistry (IJPPB) (ISSN 2141-2162) is published Monthly (one volume per year) by Academic Journals.

International Journal of Plant Physiology and Biochemistry (IJPPB) provides rapid publication (monthly) of articles in all areas of the subject such as plant hormones, seed biology, plant DNA repair, Concepts of target cells in plant differentiation 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 IJPPB are peer-reviewed.

Contact Us

Editorial Office:   ijppb@academicjournals.org
Help Desk:        helpdesk@academicjournals.org
Website:            http://academicjournals.org/IJPPB
Submit manuscript online   http://ms.academicjournals.me/
Editors

Prof Dr. Ishrak Khafagi
Faculty of Science,
Suez Canal University,
Ismailia,
Egypt

Prof. Mohamed Mahgoub Azooz
Biology Department
Faculty of Science,
King Faisal University,
Saudi Arabia.

Dr. Bansal Parveen
National Institute of Ayurvedic Pharmaceutical Research
Moti Bagh Road,
Patiala-(Punjab) India.

Prof. Bechan Sharma
Department of Biochemistry,
University of Allahabad,
Faculty of Science,
Allahabad-211002,
India.

Editor Board

Prof. Weimin Zhang
Guangdong Institute of Microbiology 100 Central Xian lie Road, Guangzhou, Guangdong 510070, China.

Dr. Xu Suxia
 Fujian Institute of Subtropical Botany, 780-800, Jiahe Road,
 Xiamen,
 China361006, China.

Dr. Adaku Vivien Iwueke
Department of Biochemistry,
Federal University of Technology,
Owerri
Nigeria.

Ass. Prof. Turgay CELIK
Gulhane Military Medical Academy,
School of Medicine,
Department of Cardiology,
Turkey.

Dr. Topik Hidayat
Department of Biology Education
Indonesia University of Education (UPI)
Jalan Dr. Setiabudhi 229 Bandung 40154 Indonesia

Dr. Tariq Mahmood
Quaid-i-Azam University,
Department of Plant Sciences,
Quaid-i-Azam University,
Islamabad,
Pakistan.

Dr. Neveen B. Talaat
Department of Plant Physiology,
Faculty of Agriculture,
Cairo University,
Egypt.

Dr. Sudhamoy Mandal
Fulbright Visiting Fellow
Department of Plant Pathology
University of Nebraska Lincoln USA
Asso. Prof. Chankova Stephka Georgieva
Central Laboratory of General Ecology, Bulg Acad
Sci 1113 Sofia, 2 Gagarin str, Bulgaria.

Shijie Han
Center of Forestry, Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang City, Liaoning Province 110016, PR China.

Szu-Chuan Shen
Department of Medical Nutrition, I-Shou University Yanchao Township, Kaohsiung County 824,Taiwan.

Seddik Khennouf
Dept of Biology, Faculty of Science University Ferhat Abbas, SETIF, 19000, ALGERIA.

Saranyu Khammuang
Department of Chemistry, Faculty of Science, Mahasarakham University, Thailand.

Samir Ranjan Sikdar
Bose Institute P-1/12, C.I.T. Scheme VII M, Kolkata 700 054, India.

Dr. M. Abdul Salam
Department of Agronomy, College of Agriculture, Kerala Agricultural University, Vellanani 695 522, Trivandrum, Kerala.

Dr. Saeed Aminzadeh

Dr. Ruzica Stricevic
Faculty of Agriculture, University of Belgrade Nemanjina 6, Zemun, 11080, Serbia.

Rumbos Christos
University of Thessaly Fytokoy Str, 384 46 Volos, Greece.

Dr. Özge Zencir
Kemah Vocational Training School, Erzincan University, Kemah, Erzincan, Turkey.

Riyazali Zafarali Sayyed
SI P Arts, GBP Science & STSKVS Comm. College SHAHADA Dist Nandurbar, Maharashtra, India.

Raul Rodriguez-Herrera
Universidad Autonoma de Coahuila School of Chemistry Blvd. V. Carranza y Gonzalez Lobo s/n Col Republica Saltillo Coahuila México.

Dr. A. H. M. Mahbub Rahman Rajshahi University, Bangladesh. Department of Botany, University of Rajshahi, Bangladesh.

Paul S. Marley
Department of Crop Protection, IAR/FOA Ahmadu Bello University, P.M.B. 1044, Samaru, Zaria, Nigeria.

Patrick Addo-Fordjour
Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science And Technology (KNUST), Kumasi, Ghana.

Battu Prasanna
Reddy Nosh Labs Pvt Ltd Hyderabad, India.
Noureddine Benkeblia
UWI - Department of Life Sciences
Mona Campus,
Kingston 7,
Jamaica.

Keutgen, Norbert
Universytet Technologiczno-Przyrodniczy
im. Jana i Jedrzej Sniadeckich w Bydgoszczy
Kadra Katedry Fizjologii Roslin
(Institute of Plant Physiology)
ul. Bernardyńska 6/8, 85-029 Bydgoszcz,
Poland.

Nicholas E. Korres
University College Cork,
Environmental Research Institute.
Lee Road, Cork,
Ireland.

Dr Naveen Kumar
University of Florida
2685 SR 29 N SWFREC/IFAS/UFL,
Immokalee, FL34142,
USA.

Dr Modala Venkateswarlu
Seribiotech research Laboratory,
Kodathi Carmelaram post,
Bangalore.

Mirza Hasanuzzaman
Department of Agronomy,
Faculty of Agriculture,
Sher-e-Bangla Agricultural University,
Dhaka-1207, Bangladesh.

Maybelle Gaballah
National Research Centre,
El Behoos street, Dokki, Cairo.

Mauro Guida Santos
Universidade Federal de Pernambuco Street
Moraes Rego – CDU – CCB – Botany Department,
s/n. 50670-901. Pernambuco State, Brazil.

Marcelo Rodrigo Pace
University of Sao Paulo Rua do
Matão, 277, Cidade Universitária São Paulo, Brazil.

Marcelo Francisco Pompelli
Federal University of Pernambuco
Department of Botany, Profª Moraes Rego Av.,
Recife – PE – Brazil, 50670-901.

Luca Catalina Mariana
University of Bucharest, Faculty of Biology, Dept
of Biochemistry and Molecular Biology
Spl. Independentei, no.91-95, Bucharest 5,
Romania.

Lin Wang
Institute of Biostatistics, Fudan University
220 Handan Road, Shanghai 200433,
China genetics, microbiology
China.

Li Qiang
Institute of karst geology,MLR
50 Qixing Road,
China.

Dr. Ayanakumar Kumar
C.Abdul Hakeem College of Engg. & Tech.,
Melivilam-632 509, Vellore Dist, Tamil Nadu,
INDIA.

P. Krishnamoorthy
P.G. AND RESEARCH DEPARTMENT OF ZOOLOGY
RAJAH SERFOJI GOVT. COLLEGE.
India.

Hare Krishna
Central Institute of Temperate Horticulture-
Regional Station,
Mukteshwar-263 138, District-
Nainital, Uttarakhand, India.

K.G. Mandal
Directorate of Water Management (formerly
Water Technology Centre for Eastern Region)
Indian Council of Agricultural Research
C.S. Pur, Bhubaneswar-751023, ORISSA, INDIA.

Dr. Jukta Adhikari
Presidency College
86/1, College Street, Kolkata – 700 073, India.
Jorge Teixeira  
*Botany Department, Faculty of Sciences, University of Porto, Edifício FC4, Rua do Campo Alegre, S/N, 4169-007 Porto, Portugal.*

Johnson Toyn Fasimirin  
*Federal University of Technology, Akure, Nigeria Department of Agricultural Engineering, FUT, P.M.B. 704, Akure, Ondo State, Nigeria.*

Joel K. Ransom  
*North Dakota State University 166 Loftsgard Hall, Department of Plant Sciences, NDSU Dept. 7670, PO Box 6050, Fargo, ND 58108-6050.*

João Claudio Damasceno de Sá  

Jalal Jalali Sendi  
*University of Guilan Department of Plant Protection, university of Guilan, Rasht, Iran.*

Iúri Drumond Louro  
*Universidade Federal do Espírito Santo Rua Horácio Andrade de Carvalho, 210, Victoria, ES, 29052-620, Brazil.*

Hong Bo Guo  
*Northwest A and F University 22 Xinong, Yangling 712100, Shaanxi, PR China.*

Harsukh P. Gajera  
*Junagadh Agricultural University Department of Biochemistry, College of Agriculture, JAU, Junagadh- 362 001, Gujarat, India.*

Hanan Abdel Fattah El-Sadawy  
*National Research Center El-Buhoth St., Dokki, Giza, Egypt.*

Assit. Prof. Azime KÜÇÜKGÜL GÜLEÇ *Tunceli University Fisheries Faculty 62000, Tunceli/TURKEY.*

Greg T. Hannig  
*DuPont 1090 Elkton Road Newark, DE 19711.*

Gilberto Santos Andrade  
*Istituto de Biotecnologia Aplicada a Agropecuária (BIOAGRO), Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, MG 36571-000, Brazil.*

Dr. T. Muthukumar  
*Department of Botany, Bharathiar University Coimbatore -641 046, Tamilnadu, India.*

Kunjupillai Vijayan  
*Institute of Plant and Microbial Biology Academia Sinica, Taipei, Taiwan-115, ROC. Taiwan.*

Badre Alam  
*National Research Centre For Agroforestry Gwalior Road, Jhansi-284003, U.P., India.*

Abeer Essam El-Din Mahmoud  
*Biochemistry Department Genetic Engineering & Biotechnology Division National Research Center El Tahrir St., El Dokki 12622, Cairo, Egypt.*

Qazi Fariduddin  
*Aligarh Muslim University Department of Botany, Aligarh 202 002, India.*

Darmawan Darma  
*Faculty of Agriculture, Andalas University Kampus Limau Manis Padang-25163, Indonesia.*

Barbara Chaves  
*Institute for Agricultural and Fisheries Research.*

Sudhamoy Mandal  
*Central Horticultural Experiment Station (ICAR) Aiginia, Bhubaneswar, PIN-751019.*

Cavit Bircan  
*Adnan Menderes University Faculty of Agriculture Department of Food Engineering 09100/Aydin/Turkey.*
Carlos Alberto Ortega-Ojeda
Central University of Ecuador. Faculty of Agriculture
Sciences. Quito, Ecuador
Calle 12 # 29 B - 78, Apto. 102 F, Unidad Residencial
Colseguros, Cali, Colombia.

Brian Wade Jamandre
National Taiwan University
Rm. 622, life science bldg., NTU, no.1, sec.4, Roosevelt rd. Taipei 10617, Taiwan (ROC).

Bita Naseri
Agricultural Research Institute
Department of Plant Protection, Agricultural Research Institute, PO Box 45195474, Zanjan, Iran.

Behzad Kaviani
Adeyemi Oluyomi Stephen Bells
University of Technology
Chemical Sciences Department, Km 8 Idiroko Road, Ota, Ogun State, Nigeria.

Ajayi Adedayo Olajide
Adekunle Ajayi University
Dept. of Microbiology, P.M.B 01, Akungba-Akoko, Ondo State, Nigeria.

Alexandre Igor Azevedo Pereira (Pereira, A.I.A.)
Universidade Federal de Viçosa, Departamento de Biologia Animal, Programa de Pós-Graduação em Entomologia. 36570-000, Viçosa, Minas Gerais State, Brazil.

Gilberto Santos Andrade
Instituto de Biotecnologia Aplicada a Agropecuária (BIOAGRO), Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, MG 36571-000, Brazil.

Pradeep. A.R., Ph.D
Seribiotech Research Laboratory
Carmelaram.P.O; Bangalore, INDIA.

Azamal Husen
University of Gondar
Department of Biology, Faculty of Natural Sciences, University of Gondar
P.O. Box #196, Gondar, Ethiopia.

Muhammad Aslam
University College of Agriculture, Bahauddin Zakariya University
Multan 60800, Pakistan.

Autumn J. Smith
Sam Houston State University, Texas.

La Sara
Haloule University
Kampus Baru Tridharma, Kendari, Southeast Sulawesi, Indonesia.

Aliyu Mohammed
Department of Human Physiology, ABU, Zaria. Nigeria.

Prof. EL-Said Ahmed AL-Sayed Ragab National Research Institute of Astronomy and Geophysics, 11421, Helwan, Egypt.

Shnoudy Anwar Bakhoun
National Institute of Oceanography & Fisheries (NIOF), Egypt.

Antonio Americo Barbosa Viana
Embrapa Recursos Genéticos e Biotecnologia PBI-LPP1
PqEB Final W/S Norte, Brasilia, DF – Brazil

Dr. Shrish Rajmalwar
National Research Laboratory for Conservation, Shirish Rajmalwar, LG Plot No. 43, Mhada colony, Wardha – 442001, (MS) India.

Dr. Amresh Chandra
Universidade Federal de Viçosa, Departamento de Biologia Animal, Programa de Pós-Graduação em Entomologia. 36570-000, Viçosa, Minas Gerais State, Brazil.
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Atul Kumar</td>
<td>GB PANT University of Agriculture &amp; Technology Department of Basic Science, College of Forestry &amp; Hill Agriculture, HILL CAMPUS, PO Ranichauri, Tehri Garhwal, Uttarakhand State, India.</td>
</tr>
<tr>
<td>Prof. Levenko Boris</td>
<td>Natl. Botanical Gardens, NAS of Ukraine 01014 Kiev, 1 Timiryazevskaya st. Ukraine.</td>
</tr>
<tr>
<td>Dr. Dionisio G. Alvindia</td>
<td>Bureau of Postharvest Research and Extension CLSU Compound, Science City of Munoz, Nueva Ecija 3120, Philippines.</td>
</tr>
<tr>
<td>Dr. Bhoopander Giri</td>
<td>University of Delhi Department of Botany, SSNC (University of Delhi) Alipur, Delhi 110036, India.</td>
</tr>
<tr>
<td>Dr. Anjuli Sood</td>
<td>University of Delhi Department of Botany, University of Delhi, Delhi-110 007, INDIA.</td>
</tr>
<tr>
<td>Dr. A. K. Verma</td>
<td>G.B. Pant University of Agriculture &amp; Technology, Pantnagar, Department of Biochemistry, College of Basic Sciences, India.</td>
</tr>
<tr>
<td>Dr. Anjana Jajoo</td>
<td>School of Life Science, Devi Ahilya University, Indore, DAVV Khandwa Road campus, Indore 452 017, M.P., India.</td>
</tr>
<tr>
<td>Dr. Deepak Ganjewala</td>
<td>Vellore Institute of Technology University 55 Thennaraam Street, Vellore-632 014 (T.N.), India.</td>
</tr>
<tr>
<td>Dr. Geetha Govind</td>
<td>Max-Planck-Institute for Chemical Ecology Hans-Knöll Straße 8, 07745 Jena, Germany.</td>
</tr>
<tr>
<td>Dr. Hossam El-Din Saad El-Beltagi</td>
<td>Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt, P.O.Box 12613 Egypt.</td>
</tr>
<tr>
<td>Prof. Dr. Md. Shahidul Haque</td>
<td>Dept. of Biochemistry and Molecular Biology University of Rajshahi, Rajshahi-6205, Bangladesh Bangladesh.</td>
</tr>
<tr>
<td>DR. P.K. NAGAR</td>
<td>Retired Senior Scientist, IHBT, Palampur, (H.P.), B.21/115-10A Batuk Dham Colony, Kamachha, Varanasi 221 010, INDIA.</td>
</tr>
<tr>
<td>Dr. Satyawati Sharma</td>
<td>Indian Institute of Technology Centre for Rural Development &amp; Technology, IIT Delhi-110016 Biomass Production on waste land, India.</td>
</tr>
<tr>
<td>Dr. Uğur Çakıcıoğlu</td>
<td>Firat University Elazığ/TURKEY Cumhuriyet M. Malatya C. No:50/A.</td>
</tr>
<tr>
<td>Prof. Abdelrhani Elachqar</td>
<td>Faculty of Sciences Dhar El Mahraz, Fez, Morocco BP 1796, Fès-Atlas, Fès, Maroc, Morocco.</td>
</tr>
<tr>
<td>Ass. Prof. Jianfeng Xu</td>
<td>Arkansas State University PO Box 639, State University, AR 72467 USA.</td>
</tr>
<tr>
<td>Ass. Prof. Jin Xu</td>
<td>Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences Huazhong RD 286, Shijiazhuang, HeBeı, China.</td>
</tr>
<tr>
<td>José Carlos Rebuglio Vellosa Ph.D</td>
<td>PARANÁ STATE UNIVERSITY OF PONTA GROSSA (Universidade Estadual de Ponta Grossa – UEPG) General Carlos Cavalcanti Avenue, 4748, Uvaranas, Ponta Grossa/PR – PO box 84030-900</td>
</tr>
<tr>
<td>Dr. Krouma Abdelmajid</td>
<td>Centre of Biotechnology, Borj Cedria Ecopark BP 901, Hammam-Lif 2050, Tunisia College of Science and Arts, Qassim University, BP 53, Al-Rass 3330353, Qassim, Saudi Arabia Saudi Arabia</td>
</tr>
<tr>
<td>Name</td>
<td>Institution</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Dr. Majid Rostami</td>
<td>Malayer University Department of Agriculture and Natural Resources, Postal code: 65719-95863, University of Malayer Malayer, Iran.</td>
</tr>
<tr>
<td>Dr. Mohammad Nasir Khan</td>
<td>Aligarh Muslim University, Aligarh, INDIA Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh-202 002, U.P., India.</td>
</tr>
<tr>
<td>Prof. N.K. Matta</td>
<td>Kurukshetra University Department of Botany, Kurukshetra University, Kurukshetra 136119, INDIA.</td>
</tr>
<tr>
<td>Dr. Naceur Djebali</td>
<td>Centre of Biotechnology Borj-Cedria (CBBBC) BP 901, Hammam-Lif 2050 Tunisia.</td>
</tr>
<tr>
<td>Dr. Nader Choparzadeh</td>
<td>Azerbaijan University of Tarbiat Moallem, Tabriz, Iran.</td>
</tr>
<tr>
<td>Nautiyal Prakash Chandra</td>
<td>Directorate Of Groundnut Research (ICAR) Post box, No. 5, Jnagadh-362001, Gujarat, India.</td>
</tr>
<tr>
<td>Prof. Hussein Fawzy Hussein Abouziena</td>
<td>National Research Center Botany Department, National Research Center, Elbhos Street, Dokki, Cairo, Egypt.</td>
</tr>
<tr>
<td>Dr. D.E. Chandrashekar Rao</td>
<td>National Research Council Canada / Plant Biotechnology Institute (NRCPB) 110 Gymnasium Place / Saskatoon, Saskatchewan S7N OW9 Canada.</td>
</tr>
<tr>
<td>Dr. S.R Madhan Shankar</td>
<td>PSG College of Arts &amp; Science Civil Aerodrome Post, Coimbatore-641 014, India.</td>
</tr>
<tr>
<td>Prof. Dr. Safdar Hussain Shah</td>
<td>Institute of Biotechnology and Genetic Engineering NWFP, Agricultural University Peshawar, Pakistan.</td>
</tr>
<tr>
<td>Prof. Dr. Md. Shahidul Haque</td>
<td>Dept. of Biochemistry and Molecular Biology University of Rajshahi, Rajshahi-6205, Bangladesh.</td>
</tr>
<tr>
<td>Dr. Sivakumar Swaminathan</td>
<td>Iowa State University (ISU) G-319, Agronomy Department, ISU, Ames, Iowa - 50011, USA.</td>
</tr>
<tr>
<td>Dr. Subrahmanyam Desiraju</td>
<td>Directorate of Rice Research (ICAR) Plant Physiology Division, Rajendranagar, Hyderabad-500030, A.P. India.</td>
</tr>
<tr>
<td>Dr. Tariq Aziz Dr. Deepak Ganjewala</td>
<td>University of Agriculture, Faisalabad, Sub-Campus Depalpur, Dist. Okara, Pakistan.</td>
</tr>
<tr>
<td>Dr. Thangavel Palaniswamy</td>
<td>YAT-SEN UNIVERSITY GUANGZHOU, PR CHINA.</td>
</tr>
<tr>
<td>Yi-Ping Chen Ph.D</td>
<td>Institute of Earth Environment, Chinese Academy of Science Fenghui S.R, 10, Xi’an Hi-Tech Zone, Xi’an, China.</td>
</tr>
<tr>
<td>Saha Prasenjit</td>
<td>The Samuel Roberts Noble Foundation 2510 Sam Noble Parkway, Ardmore, Ok USA.</td>
</tr>
<tr>
<td>Abdul Khaliq Ph.D</td>
<td>Department of Agronomy University of Agriculture Faisalabad 38040, Pakistan.</td>
</tr>
<tr>
<td>Dr. Arafat Abdel Hamed abdel Latif</td>
<td>Assistant Professor of Plant physiology Botany Department Faculty of Science at Qena South Valley University Egypt.</td>
</tr>
<tr>
<td>Dr. Ahmad Bybordi</td>
<td>Research Center of Agriculture and Natural Resources of East Azerbaijan Member of Scientific Board of Research Center of Agriculture and Natural Resources of East Azerbaijan, Tabriz, Iran.</td>
</tr>
</tbody>
</table>
Dr. Arijit Sinhababu  
Bankura Christian College (under –The University of Burdwan)  
Department of Botany, Bankura Christian College, P.O. + Dist. Bankura, Pin.-722101, West, Bengal, India.

Dr. Maria Alejandra Equiza  
University of Alberta, 4-51 Earth Sciences Building, Dept. Renewable Resources, University of Alberta, Edmonton, AB T6G 2E3, Canada

Dr. Suphla Bajpai Gupta  
Indian Institute of Integrative Medicine –CSIR, Scientist, Plant biotechnology division, Canal Road, Jammu, Jammu & Kashmir, India-180001

Dr. Linga R Gutha  
Washington State University, 2410 N Bunn Road, Prosser, WA 99350, USA.

Dr. Medhat Mekhail Tawfik  
National Research Center, El Bohooth Str. Dokki, Giza. Egypt, PO Box 12311, Egypt.

Dr. Rafaq Islam  
The Ohio State University South Centers, 1864 Shyville Road, Piketon, OH 45661.

Dr. Rakesh Kumar  
V.S.P. Govt. P.G. College, Kairana, Muzaffarnagar (Uttar Pradesh), Department of Botany, V.S.P. Govt. P.G. College, Kairana, Muzaffarnagar (Uttar Pradesh), India-247774.

Dr. Ivan Sestari  

D.Sc. Rachel Fatima Gagliardi  
State University of Rio de Janeiro, Rua São Francisco Xavier, 524 – PHLC sala 602.

Dr. Ullas Pedmale  
Salk Institute for Biological Studies, 10010 N Torrey Pines RD, La Jolla, CA 92037.

Dr. Allah Bakhsh Dr. Deepak Ganjewala  
Department of Field Crops, Faculty of Agriculture, University of Ankara, Apartment No. 12/10, Sanatorym Caddesi, Kalaba, Kecioren, Ankara, Turkey.

Dr. Atilgan Atilgan  
Suleyman Demirel University, Agriculture Faculty, Department of Agricultural Structures and Irrigation, Isparta, Turkey.

Mr. Andrej Pilipovic  
University of Novi Sad – Institute of Lowland Forestry and Environment, Antona Cehova 13, 21000 Novi Sad, Serbia.

Dr. Zulficar Ahmad Saqib  
Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Civil Line Road, Faisalabad, Pakistan.

MS. C. Mehnoush Eskandari Torbaghan  
North Khorasan Agricultural & Natural Resource Research Center (NKANRRC) P.O. Box: 94155-1416, No. 52, Hassan Kallate Alley, Tarbiyat St., Mother Sq. Bojnourd, Iran.

Dr. Vinod Kumar  
Department of Zoology & Environmental Science, Gurukula Kangri University, Haridwar-249404 (UK), India.

Dr. Panda Tribhubana  
Kalahandi Institute for Tribiology and Ethnobiology(KITE), At-Jilingdar, PO-Deydar, Dist-Kalahandi,Odisha, India 766014, India

Dr. Sabarinath Sundaram  
Institute of Developmental and Molecular Biology, Texas A&M University, Biological Sciences Building West Suite 403.
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Diogo Pineda Rivelli</td>
<td>University of São Paulo, Av. Prof. Lineu Prestes 580, São Paulo, SP, 05508-000.</td>
</tr>
<tr>
<td>Dr. Qiang Wang</td>
<td>Virginia Tech, 427 Latham Hall.</td>
</tr>
<tr>
<td>Dr. Foteini Hassiotou</td>
<td>University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.</td>
</tr>
<tr>
<td>Dr. Nivedita Sahu</td>
<td>Indian Institute of Chemical Technology, Chemical Biology Laboratory (NaturalProductChemistry), Uppal Road, Hyderabad-500607.</td>
</tr>
<tr>
<td>Dr. Mohammad Anwar Hossain</td>
<td>Bangladesh Agricultural University, Assistant Professor, Dept. of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.</td>
</tr>
<tr>
<td>Dr. Ahmad Ali</td>
<td>National Institute of Pharmaceutical Education &amp; Research, Dept of Biotechnology, NIPER, Jandaha Road, Hajipur, Bihar, India, Pin – 844102, India.</td>
</tr>
<tr>
<td>Mr. Karthik Kumar V</td>
<td>Annamalai University, Department of Biochemistry &amp; Biotechnology.</td>
</tr>
<tr>
<td>Dr. K.Rajendiran</td>
<td>Dept of plant science, Tagore Govt. college, 9, 4th cross, Tagore Nagar, Pondicherry – 605 008, India.</td>
</tr>
<tr>
<td>Dr. V. Balakrishnan</td>
<td>K.S.Rangasamy College of Technology, Department of Biotechnology,KSR Kalvi nagar,Tiruchengode- 637215,Tamilnadu, India.</td>
</tr>
<tr>
<td>Dr. NourAli Sajedi</td>
<td>Department of Agronomy and plant Breeding, Islamic Azad University, Arak Branch, Arak, Iran.</td>
</tr>
<tr>
<td>(Dr) Ms. Rachel Predeepa</td>
<td>Not Applicable , 2/387 Gokul Nagar, Kannanenthal Madurai.</td>
</tr>
<tr>
<td>Dr. Rajendra Gyawali</td>
<td>Department of Pharmacy and Biology, Kathmandu University, Dhulikhel, Nepal.</td>
</tr>
<tr>
<td>Ms. Rocheli de Souza</td>
<td>UFRGS, Porto Alegre, Brazil.</td>
</tr>
<tr>
<td>Dr. Om Prakash Verma</td>
<td>Sam Higginbottom Institute of Agriculture, Technology &amp; Sciences (Formerly Allahabad Agricultural Institute), Allahabad, U.P., Department of Molecular &amp; Cellular Engineering, Jacob School of Biotechnology &amp; Bioengineering, India.</td>
</tr>
<tr>
<td>Dr. Ashwani Kumar</td>
<td>JMIT, Radaur, Department of Biotechnology, JMIT, Radaur-135133, Haryana, India.</td>
</tr>
<tr>
<td>Dr. Sarfaraz F. A. Al-Bamarny</td>
<td>University of Duhok, College of Agriculture, Dept. of Horticulture, Duhok, Iraqi Kurdistan Region, Iraq.</td>
</tr>
<tr>
<td>Prof. Wafaa Mohamed Shukry Abdel Meamem</td>
<td>Dammam University - Saudi Arabia, Faculty of Science for Girl. Biology Department, P. O.Box: 838 Dammam 31113, Saudi Arabia.</td>
</tr>
<tr>
<td>Dr. Stephka G. Chankova</td>
<td>Institute of Biodiversity and ecosystem Research, BAS, 2 Gagarin str, 1113 Sofia, Bulgaria.</td>
</tr>
</tbody>
</table>
Dr. Nana Ewusi-Mensah  
*Kwame Nkrumah University of Science and Technology,  
Dept. of Crop and Soil Sciences, Faculty of Agriculture, KNUST, Kumasi.

Dr. Mukesh Lokanath Chatan  
*K.r.c. College of horticulture, arabhavi 591 310, karnataka,  
University of horticultura sciences, bagakot, India.

Dr. MaiTai Parthapratim  
Dept. of Botany Midnapore College, Midnapore-721101, Paschim Medinipur, West Bengal, India.

Mr. Mohammad Anwar Hossain  
Kagawa University (Present), Bangladesh Agricultural University (Permanent)  
Lab. of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Miki-cho, Kitagun, Kagawa 761 0795, Japan.

Dr. Antonia Tathiana Batista Dutra  
Universidade Federal do Ceará, Av. Humberto Monte s/n – Pici Bloco 907, laboratório 1080.

Dr. Kuntal Das  
St. John’s Pharmacy College,  
#6, II Main, 9th Cross, Vijayanagar, Bangalore-104, India.

Dr. Amitava Rakshit  
Banaras Hindu University,  
Department of Soil Science & Agril Chemistry.

Dr. Kranthi Kiran Mandadi  
Texas A&M University,  
2132 TAMU, Peterson-Rm408, College Station, Texas-77840, USA.

Dr. Monica Butnariu  
*Banat’s University of Agricultural Sciences and Veterinary Medicine from Timisoara, Chemistry and Vegetal Biochemistry Department, Calea Aradului no.119, 300645 Timisoara, Romania.

Dr. Ahmad Bybordi  
East Azarbaijan Research Center for Agriculture and Natural Resources, Tabriz, Iran.

Dr. Haiwei Gu  
903 Fifth St., West Lafayette, IN 47906.

Dr. Hu Yanbo  
Northeast Forestry University,  
26# Hexing Road, Xiangfang District, Harbin city,  
150040, P.R., China.

Dr. Arash Kianianmomeni  
Institute of Biology / Humboldt-University Berlin, Invalidenstr. 42.

Dr. Zvonko Pacanski  
Faculty for Agriculture Sciences and Food, Boul. Aleksandar Makedonski bb,  
1000 Skopje, R.of Macedonia.

Dr. Lingjuan Zheng  
Department of Organismic Biology, University of Salzburg, Hellbrunnerstraße 34, 5020, Salzburg, Austria.

Dr. Md. Mokter Hossain  
Department of Horticulture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Dr. Forouzande Soltani  
Department of Horticultural Sciences, College of Agriculture and Natural Resources, University, of Tehran, Daneshkadeh Street, Karaj 31587-11167, Iran.

Dr. M.C.Harish  
Bharathiar University,  
Department of Biotechnology, Coimbatore, India.

Dr. Zong-shen Zhang  
School of Biological Engineering, Dalian Polytechnic University,  
Qinggongyuan, Ganjingzi District, Dalian, China, postcode 116034.
Prof. T. V. Ramana Rao  
B R Doshi School of Biosciences,  
Sardar Patel University, Vallabh Vidyanagar,  
Gujarat, India.

Dr. Sanjeev Chandel  
Baba Isher Singh Institute of Sciences & Technology, Gagra (Moga), Punjab, India.

Dr. Kuladip Jana  
Bose Institute Centenary Campus, P 1/12, C.I.T.  
Scheme VIIIM, Kolkata-700 054, India.

Prof. Ljubinko Jovanovic  
University Educons,  
Faculty for Ecological Agriculture, Sremska Kamenica, Vojvode Putnika 87, Serbia.

Dr. Luis F. Goulao  
Instituto de Investigacao Cientifica Tropical  
[Tropical Research Institute] Eco-Bio / IICT, Av. da Republica - Quinta do Marques, 2784-505 Oeiras,  
Portugal.

Dr. Lucky K. Attri  
College of Punjabi University Patiala, E-41,  
Sector-14, Panjab University, Chandigarh.

Prof. Bassam Taha Yasseen  
Flat 307 Point Red,  
146 Midland Road, Luton, LU2 0BL, UK.

Dr. Massimo Piccotto  
Tecna S.r.l.,  
Area Science Park, Loc. Padriciano, 99, I-34149 Trieste, Italy.
ARTICLE

Influence of the position of flowers buds on the tree on somatic embryogenesis of cocoa (*Theobroma cacao* L.)

Rodrigue Pouengue Bouthouang, Olive Flore Zebaze Akitio, Audrey Germaine Ngouambe Tchouatcheu, and Nicolas Niemenak
Full Length Research Paper

Influence of the position of flowers buds on the tree on somatic embryogenesis of cocoa (*Theobroma cacao* L.)

Rodrigue Pouengue Boutchouang¹,², Olive Flore Zebaze Akitio²,³, Audrey Germaine Ngouambe Tchouatcheu²,³ and Nicolas Niemenak²

¹Department of Biochemistry, Faculty of Sciences, University of Yaounde I, P. O. Box 812, Yaounde-Cameroon.  
²Laboratory of Biochemistry and Plant Physiology, Department of Biological Science, Higher Teachers’ Training College, University of Yaounde I, P. O. Box 47, Yaounde- Cameroon.  
³Department of Plant Biology, Faculty of Science, University of Yaounde I, P. O. Box 812 Yaounde, Cameroon.

Received 22 February, 2016; Accepted 26 May, 2016

The recalcitrance of *Theobroma cacao* L. to somatic embryogenesis, due to non-adapted physiological and metabolical responses to environmental stress, limits its propagation. The present work aims to ameliorate somatic embryogenesis in *T. cacao* throughout a physiological approach. For this purpose, the influence of the position of flowers buds used as explants was evaluated. Flowers buds were collected from different parts of the tree: orthotropic main stem (OS), primary plagiotropic fan branch (FI) and secondary plagiotropic fan branch (FII). Evolution of some biochemical parameters such as phenolic compounds, soluble sugars, proteins content and peroxidase activity was followed at different steps of somatic embryogenesis, considering the origin of the explants used. Results obtained show that callogenesis is induced on all explants independently of their origin, with an 80% average frequency. Embryogenesis frequencies were ca 2 fold higher in staminodes-derived calluses from FII and FI than OS. Meanwhile petals of FII do not differentiate embryos. Biochemical analysis shows that the content of phenol is low in calluses during somatic embryo establishment. Explants from FII present the lowest values (after 49th days of culture). Sugars content decrease during callogenesis. When embryos are established the sugars content decrease in explants from OS. During the same period, proteins’ and phenols contents increased in staminodes-derived calluses from all origin; while there was decrease in petals from FI and FII. Buds from fan branch are suitable for somatic embryogenesis process and this capacity correlate with peroxidase activity which decrease during embryos dedifferentiation phase.

Key words: *Theobroma cacao* L., somatic embryogenesis, proteins, phenols compounds, soluble sugars, peroxidase activity, microclimate.

INTRODUCTION

*Theobroma cacao* L. (chocolate tree) is grown in the humid tropics and constitutes an important source of incomes for many countries of the West and Central Africa regions. This tree, when grown in wild state, can...
reach 12 to 15 m of length (Wood et al., 1985). It is a diploid (2n=20) and preferentially allogamy plant. The root system has a growth dimorphism characterized by an orthotropic development axis, the pivot and plagiotropic lateral ramifications and lateral roots. Cocoa trunk is characterized by a vertical harbor (orthotropic), a phyllotaxis 3/8; long-stalked leaves, axillary orthotropic buds, a defined growth, differentiation of three to five plagiotropic buds under the apex, at the time of the degeneracy of the terminal bud. Caulifloriferous tree, inflorescences cymes biparous with reduced inter-node develop at the flowering areas called flower cushions. The flowers are hermaphroditic, with small sizes (diameters ranging from 0.5 to 1 cm), regular and composed of 5 sepals, 5 petals, 5 staminodes, a pistil and an ovary. Pollination is predominantly entomophilous although it can be done manually in the experimental fields. Flowering in cocoa is manifested by the production of a minimum of 50 000 flowers during the term with less than 5% of production pods (Lass, 1999). Cocoa trees demonstrate a high degree of segregation for many traits when propagated by seeds. For this reason, clonal propagation systems such as rooted cuttings and grafting have been applied for multiplication of elite varieties but a vast majority of cocoa plants in production were derived from seeds (Eskes, 2005). The use of in vitro propagation methods for cocoa could potentially contribute to efforts at crop improvement, germplasm conservation, and rapid distribution of new improved varieties.

Somatic embryogenesis is a vegetative propagation method which permits the production of several embryos capable of generating plants similar to the initial one from non-sexual tissues. Somatic embryogenesis has been the most used method these last years for in vitro regeneration of elite genotypes of cocoa (Niemenak et al., 1998; Maximova et al., 2008; Noah et al., 2013). Somatic embryos germinate to yield entire plant with a pivoting root that we cannot obtain by cutting. Temporary immersion culture and suspension culture can enhance embryos multiplication of cocoa (Niemenak et al., 2008). The success of this method is mostly dependent of genotype and culture medium composition. Li et al. (1998) achieved better somatic response from many genotypes using DKW complex salt with floral explants. Somatic embryogenesis plays an important role in clonal propagation. When integrated with conventional breeding programs and molecular plus cell biological techniques, somatic embryogenesis provides a valuable tool to enhance the pace of genetic improvement of commercial crop species (Stasolla and Yeung, 2003). There are evidences that the main metabolic and developmental processes occurring in the zygotic embryogenesis may be recapitulated in the somatic embryogenesis (Fehér et al., 2003). But the latter are less efficient in converting carbohydrates in lipids and storage proteins during the late developmental stages (Cangahuala et al., 2009). The levels of these substances change along the developmental stages of cells cultures, and their role has been ascribed to the transduction signal cascade or as substrate for cell growth and morphogenesis (Lulsdorf et al., 1992). Storage proteins are the source of amino acids for seed germination (Misra et al., 1993). Proteins could also be involved in the regulation of cell expansion and establishment of biophysical characteristics required for the morphogenesis (Jiménez, 2001).Soluble sugars, such as glucose and sucrose, are involved in the regulation of developmental processes occurring from embryo development to seed maturation (Gibson, 2005).

Generally, when used as source of plant materials for somatic embryogenesis, flowers buttons are collected from the tip of branches on cocoa trees, whereas their production is distributed on the whole plant and is extended all over the year. Due also to the low production and conversion of somatic embryos obtained from different genotypes of T. cacao, leading to important economic lost, a new physiological approach was developed. Assessment of somatic embryogenesis was done using floral explants (staminodes and petals) from different position on the tree: orthotropic main stem (OS), primary plagiotropic fan branch (starting from the first ramification) and secondary plagiotropic fan branch (starting from the second ramification). The present research work objectives were to investigate the mechanism that can help to solve the recalcitrance of T. cacao and to understand the influence of explant position on the tree on biochemical events underlying somatic embryogenesis in this specie.

MATERIALS AND METHODS
Preparation of plant materials

The tissue system used in this investigation was the same as the system previously described by Minyaka et al. (2008). Studies were carried out on the cacao genotype “SCA 6”, which is included in the gene-bank of the Institute of Agricultural Research and Development at Nkolbisson (Yaounde, Cameroon). Flowers buds were collected at different position on the same tree during all the experimentation, including orthotropic main stem (OS), primary plagiotropic fan branch (FI) and secondary plagiotropic fan branch (FFI) early in the morning. They were surface sterilized by immersion for 20 min in 3% (w/v) sodium hypochlorite followed by three rinses in sterilized distilled water of 2 min each. Staminodes and petals were excised with scalpels and placed on culture media (in distinct set) into Petri dishes (Figure 1).

Culture medium preparation

All media were defined using Driver and Kuniyuki Walnut (DKW) medium basal salt of Driver and Kuniyuki (1984). The explants were first cultured in primary callus growth medium. Primary callus growth (PCG) medium was supplemented with 250 mgL\(^{-1}\) glutamine, 100 mgL\(^{-1}\) myo-inositol, 1 mL\(^{-1}\) DKW vitamin stock (100 mgmL\(^{-1}\), 2 mgmL\(^{-1}\) thiamine-HCl, 1 mgmL\(^{-1}\) nicotinic acid and 2 mgmL\(^{-1}\) glycine), 20 gL\(^{-1}\) glucose, 18 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 45.4 nM thidiazuron (TDZ). Media were dispensed into sterilized Petri dishes after autoclaving for 20...
min at 1 bar pressure and 121°C. Each Petri dish contains either 30 staminodes or 30 petals. Experiments were repeated 5 times with five replicate Petri dishes at each culture initiation. Petri dishes were incubated in dark at 25 ± 1°C for 14 days. After 14 days in PCG medium, explants were transferred to secondary callus growth (SCG) medium. SCG medium consisted of DKW basal salts, supplemented with 0.5 mL⁻¹ DKW vitamin, 20 g L⁻¹ glucose, 9 μM 2,4-D, 250 μgL⁻¹ kinetin and 0.22% (w/v) gelrite. Cultures were also incubated at 25 ± 1°C for 14 days in darkness. Cultures from SCG medium were transferred in embryo development (ED) medium. ED medium was made of DKW basal salts supplemented with 6.0 mM MgSO₄, 1 mL DKW vitamin, 30 g L⁻¹ sucrose, 1 g.L⁻¹ glucose and 0.22% (w/v) gelrite. Cultures were incubated at 25 ± 1°C in darkness for 21 days. Two others sub-cultures of explants were made every 21 days in ED medium for the development of embryos.

**Biochemical analysis**

At the end (91st days) of each experience (a given culture), calluses of each characteristic development stages of somatic embryogenesis, that is, calluses aged 14, 28, 49, 70 and 91 days, respectively, were collected from different sources of explant and analyzed independently.

**Estimation of phenolic contents**

Phenolic compounds were extracted as described by El Hadrami (1995) and Macheix et al. (1990). Embryonic mass (100 mg) were ground in chilled mortars with 2 ml of 80% (v/v) methanol at 4°C. After incubation, tubes were centrifuged thrice at 7000 g for 30 min, supernatant were recuperated each time. Mixture of the three supernatants constituted the crude extract. Total phenols were quantified using the method described by Singleton and Rossi (1965). 15 μl of alcoholic extract were added to Folin-Ciocalteu reagent (250 μl), 2.5 mL of distilled water and 0.5 ml of sodium carbonate (20 %). The mixture was incubated at 40°C for 20 minutes and the blue color was determined at 760 nm. The content of soluble phenolic was expressed in mg-equivalent of gallic acid per fresh weight (FW).

**Estimation of soluble sugars contents**

Soluble sugars were extracted according to the modified method of Babu et al. (2002). Biological material (400 mg) was ground in mortar with 2 mL of 80% (v/v) ethanol and centrifuge at 6000 g for 20 min. The supernatant was collected and constituted the crude extract. 50 μL of this alcoholic extract was added to 5 mL of Anthron reagent, homogenized and incubated at 80°C for 20 min.
Table 1. Average frequency of callogenesis in Sca 6 genotype of *Theobroma cacao* L., 28 days after induction in primary callus growth (PCG) medium.

<table>
<thead>
<tr>
<th>Calogenic explants</th>
<th>Orthotropic main stem</th>
<th>Primary plagiotropic fan branch</th>
<th>Secondary plagiotropic fan branch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staminodes-derived callus (%)</td>
<td>92.50 ± 4.97a</td>
<td>93.97 ± 2.43a</td>
<td>81.23 ± 6.40a</td>
</tr>
<tr>
<td>Petals-derived callus (%)</td>
<td>83.47 ± 9.87a</td>
<td>90.17 ± 5.00a</td>
<td>93.63 ± 1.78a</td>
</tr>
</tbody>
</table>

Each value is mean ± SE of three replicates samples. Values significantly different at the 5% level of significance are indicated with different letters.

After cooling in melting ice, the absorbance of the green complex formed was determined at 620 nm. Glucose was used as standard.

### Estimation of protein contents and POX activity

Calluses (500 mg) were ground in chilled mortar with 2 mL of TAMET buffer (0.5 M Tris, 0.3 M ascorbic acid, 0.2% (v/v) β-mercaptoethanol, 0.01 M EDTA and 0.02% (v/v) Triton X 100, pH 6.7); and 0.125 g polyvinylpyrrolidone was added in the medium. The crude homogenate was centrifuged for 15 min at 20000g and 4°C. The supernatant was removed and used as crude extract for proteins and enzymes assays. Proteins were quantified according to Bradford (1976) method. Peroxidase (POX) activity was measured spectrometrically at 420 nm by the guaiacol-H₂O₂ method of Erdelsky and Fric (1979). The specific activity of the enzymes was expressed as the change in optical density per mg of fresh weight (FW).

### Statistical analysis

Data were subjected to statistical analysis using SPSS software version 16.0. Analysis of variance was performed where applicable and differences between means were determined using LSD and Tukey Test.

### RESULTS

The results showed that calluses were distinguishable on floral explants after 4 to 5 days of cultivation in the primary growth medium. Callus development was well established in secondary callus growth medium, and was quite similar in tissues from different origins, according to their morphological aspects (Figure 1). There were no significant differences (p<0.05) among the average frequency of callogenesis (after 28 days) when explants were cultivated in DKW medium containing plant regulators. However, callus growth was most influenced by the type and origin of explant used. The average callogenesis frequency was up to 80% in *T. cacao* tissues, with the highest value obtained with staminodes explants collected on FI (93.97±2.43%) (Table 1). By the end of three-week in embryo development medium, some calluses differentiated roots or embryos. The earliest somatic embryos were observed between 49 and 55 day of culture on embryogenic calluses. Petals-derived calluses collected on secondary branches do not differentiate somatic embryos during all the experiment (91 days). The percentage of explants producing somatic embryos is highly influenced by the nature and the origin of explant cultivated in culture media (Figure 2). No statistical differences (p<0.05) were found among the rate of petals-derived calluses producing somatic embryos from different origin. Whereas, in staminodes-derived calluses, this rate decrease significantly when moving up from OS to FII. In fact, this rate of explants-derived calluses producing somatic embryos were approximately 3 times higher in staminodes tissues than petals after 91 days of culture, with the highest value obtained with staminodes-derived calluses from FII (13.70±3.21%). As observed, embryogenic and non-embryogenic tissues of *T. cacao* calluses were discernable based on coloration; embryogenic calluses were brown (phenolized) and friable, while non-embryogenic were white and rough. Somatic embryos undergo different developmental stages as shown by the result: globular, heart shaped, torpedo and cotyledonary stage (Figure 1C, D, E and F). Cotyledonary stage constituted the last development stage. At this stage, somatic embryos submitted to maturation treatment were able to germinate and generate plantlets (Figure 1G, H, and I).

Biochemical analysis showed that the total content of polyphenols compounds in staminodes-derived calluses ranged from 2.16 ± 0.15 to 8.04 ± 0.22 mg g⁻¹ of FW in OS, 1.69 ± 0.08 to 6.61 ± 0.49 mg g⁻¹ of FW in FI and from 1.73 ± 0.23 to 4.9± 0.33 mg g⁻¹ of FW in FII (Figure 3). There is no regular evolution of this determinant during somatic embryogenesis process in both types of explants (staminodes or petals) from the same position on the tree. After 28th days (corresponding to induction step), total phenols compounds increased in staminodes-derived calluses (84, 21 and 43%, for OS, FI and FII, respectively). Constitutively, soluble carbohydrates were low in calluses from all origins (Figure 4). Statistical differences (p<0.05) were found in the amount of carbohydrates between OS and FII calluses after 28th days. Whereas no statistical difference were found between the amount of sugars in calluses from FI and FII during this period, considering staminodes and petals tissues. Callogenesis in this case was characterized by a decrease in carbohydrates content. Among petals-derived calluses, those from FII displayed the highest amount of carbohydrates (6.37±0.3 mg g⁻¹ of FW after 49 days), and OS the lowest (1.6±0.18 mg g⁻¹ of FW after 28 days); While decreasing progressively in others stages.
Figure 2. Influence of the position of flowers buds on the percentage of explants of *T. cacao* producing somatic embryos after 91st days. Data are presented as means of five identical experiments; position of flowers buds: Orthotropic main stem (OS), primary plagiotropic fan branch (FI) and secondary plagiotropic fan branch (FII). Values significantly different at the 5% level of significance are indicated with different letters.

between 0.8 and 36% (in 70-day-old OS and 90-day-old FI, respectively). Except for calluses from FI and FII aged 14 days, the same observation was made in petals. The protein contents in staminodes-derived calluses varied from 0.537±0.178 mg g⁻¹ (FII after 28 days) to 2.181±0.372 mg g⁻¹ of FW (OS after 70 days), whereas in petals-derived calluses, it varied from 0.724±0.03 mg g⁻¹ (OS after 70 days) to 1.8495±0.237 mg g⁻¹ of FW (OS after 28 days). Protein content was significantly higher in petals-derived calluses from FII aged 49 days (1.809 ± 0.415 mg g⁻¹ of FW) than in OS (0.911 ± 0.178 mg g⁻¹ of FW) from the same source. It appears that, cells differentiation is characterized by high protein synthesis in staminodes tissues (Figure 5).

Peroxidases activities presented pattern related to a source of morphogenetic structure. The activity in staminodes-derived calluses after 49 days was 2.7 and 2.0 fold higher in OS than in FII and FI, respectively. There was no significant difference (p<0.05) among peroxidases activities in staminodes-derived calluses at any developmental stage of this experiment from each source of tissues. However, this value, after 28 days, was significantly higher in petals-derived calluses from BII as compared to OS and FI (Figure 6). Staminodes-derived calluses from OS presented the lowest activity (after 70 days).

**DISCUSSION**

Somatic embryogenesis is the process by which somatic cells, under inductive conditions, generate embryogenic cells, which undergo a series of morphological and biochemical changes resulting in the formation of somatic embryos (Schmidt et al., 1997; Komamine et al., 2005; Businge et al., 2013). Somatic embryogenesis forms the basis of cellular totipotency that is unique to higher plants. Currently, this clonal technique is considered to represent a prominent *in vitro* regeneration system for cocoa. Somatic embryogenesis allows rapid regeneration of elite genotypes, germplasm conservation and genetic transformation system (Maximova et al., 2002). Unfortunately, the recalcitrance of *T. cacao* to this technique and numerous factors that control it limit its systematical exploration. In this study, a comparative approach was applied to study physiological differences among *T. cacao* explants-derived calluses from different origins with the hope to understand the complexity of this phenomenon (recalcitrance). The influence of the position of flowers buds on the tree on somatic embryogenesis of “Sca 6” genotype, known as highly productive in farm was analyzed. The variation of phenols compounds, total soluble sugars, proteins contents and peroxidases activities was also performed in these conditions.

Results showed that both explants types used (staminodes and petals) are favorable to callogenesis with an average frequency above 80% in all callus-derived explants used in this experimentation. These results are in agreement with those previously obtained by Li et al. (1998) and Minyaka et al. (2009). These authors showed that callogenesis was effective in *T. cacao* using floral explants. The action of 2,4-dichlorophenoxyacetic acid (2,4-D) and that of thidiazuron (TDZ) are responsible for this callogenesis. A synergic and/or a
complementary effect of auxin/cytokinin action in induction of somatic embryogenesis process were reported in many species such as Ricinodendron heudelotti (Fotso et al., 2007) and Vitis vinifera (Olah et al., 2009). This callogenesis success could be due to the high mobilization of soluble sugars during this development step. This might consolidate the fact that reducing carbohydrates are important for calluses formation and cell differentiation (Ana et al., 1997). They regulate osmotic pressure (Blanc et al., 1999) and are major components of cell wall. In cocoa, the use of these metabolites is origin-dependent, as demonstrated by this study. And this could be justified by enzymatic equipment which varies in composition and/or function in cells of flowers buds according to their position on the tree. Study done by Alemano et al. (2003) showed that flowers buds of cocoa contain different types of phenolic compounds, and each type may be expressed qualitatively and quantitatively according to the developmental stage, indicating the key role played by these metabolites in the regulation of cell differentiation events.

Somatic embryogenesis in T. cacao is nature-and origin-dependent as shown by the present study data, but the last factor seems to be the most important. In fact, the average frequency of calluses producing somatic embryos increased when we move up from the orthotropic main stem to the secondary plagiotropic fan branch (with a maximum in staminodes-derived calluses from FII). Except for flowers buds from FII, the same observation is made for calluses of petals, as these last do not differentiate somatic embryos. Thus, somatic embryogenesis is an environmental stress-response
Figure 4. Influence of the position of flowers buds on the tree on the soluble sugars content during somatic embryogenesis. Staminodes (A) and petals (B); position of flowers: orthotropic main stem (OS), primary plagiotropic fan branch (FI) and secondary plagiotropic fan branch (FII). Vertical bars represent standard error. Each plot was drawn from means of four identical experiments.

Figure 5. Influence of the position of flowers buds on the tree on proteins content during somatic embryogenesis. Staminodes (A) and petals (B); position of flowers: orthotropic main stem (OS), primary plagiotropic fan branch (FI) and secondary plagiotropic fan branch (FII). Vertical bars represent standard error. Each plot was drawn from means of four identical experiments.
Figure 6. Influence of the position of flowers buttons on the tree on peroxidases activities during somatic embryogenesis. Staminodes (A) and petals (B); position of flowers: orthotropic main stem (OS), primary plagiotropic fan branch (FI) and secondary plagiotropic fan branch (FII). Vertical bars represent standard error. Each plot was drawn from means of four identical experiments.

process, imposed on culture medium, and this response depends on the expression of genes present in tissues (Johnson et al., 1997). This low rate of somatic embryos produced can be explained by the recalcitrance of this crop to somatic embryogenesis process.

Soluble sugars content was high in calluses from all origins during embryos dedifferentiation step in primary and secondary plagiotropic fan branches. This increase could be due to the important role play by carbohydrates metabolism in organogenesis and morphogenesis. During embryos differentiation steps, this phenomenon is progressively inversed. Moreover during callogenesis, there is a decrease of carbohydrates content in flowers buds suggesting an important utilization of these metabolites. Zygotic and somatic embryos both highly accumulate enzymes of carbohydrate metabolism as demonstrated by several studies (Iraqi et al., 2001; Hendriks et al., 2003; Noah et al., 2013). The explanation for extensive carbohydrate metabolism is the heavy energy demand required for processes that occur during cell division and elongation. One of the factors generally considered as responsible for in vitro recalcitrance of
cocoa is the high polyphenol compounds content and their oxidation. In this experiment, a high accumulation of these metabolites was particularly observed during calllogenesis and embryos differentiation steps (from 70th to 91st day). Each type of explants used expressed a significant decrease in phenols compounds during embryos dedifferentiation stage (after 49th day) when grown in DKW medium supplemented with sulphate, with the lowest content reported in petals-derived calluses from secondary plagiotropic fan branch. There are several internal and external factors affecting the quality and/or the quantity of polyphenol compounds in plants. The quantitative differences registered within explants-derived calluses from different source could be explained, at least in part, by the interaction of several genetic, physiological, agronomic (position of flower bud on the tree), and environmental factors (microclimate) modifying the final concentration in each flower (Roubelakis-Angelakis and Kliewer, 1986). Besides light and temperature (Wang and Zheng, 2001), the availability of plant nutrients also has a great influence on the accumulation of polyphenols (Francis and Atwood, 1961; Doak and Miller, 1968; Piccaglia et al., 2002). Finally, a high accumulation of polyphenol compounds in floral explants during induction steps has been demonstrated to be not favorable to somatic embryogenesis process. A similar result was obtained in date palm (*Phoenix dactylifera L.*) by Zouine and El Hadrami (2004). Peroxidase activity was high in calluses during induction steps of somatic embryogenesis and low during dedifferentiation step (after 49th day of subculture). This result might underline the implication of this peroxidase in somatic embryogenesis. In fact, the controversy of the higher peroxidase activity during dedifferentiation step of somatic embryogenesis in calluses of petals could justify their low embryos production capacity as compared to their staminodes counterpart.

In conclusion, the data presented here clearly demonstrate the efficiency of floral explants from different position on the tree to somatic embryogenesis process. The comparison between flowers buds carried by the secondary fan branch, commonly used to regenerate *T. cacao* plantlets, and those from the main stem and primary fan branch showed many differences in physiological responses. The most pronounced difference among the tree types of explants-derived calluses concern with carbohydrates metabolism: FII and FII biochemical evaluation displayed a low utilization of carbohydrates, while OS explants are characterized by intensive glycolytic activities as documented by the exceptional decreased of sugars content during somatic embryogenesis. The absence of embryos dedifferentiation on petals-derived calluses from FII has been connected, at least in part, to changes in specific enzymes abundances. The results suggest that stress factors (microclimate) and genetic factors affect embryogenic capacity of floral explants in *T. cacao* and thereby reduce the regeneration frequency.

**Conflict of Interests**

The authors have not declared any conflict of interests.

**REFERENCES**


