International Journal of Plant Physiology and Biochemistry

Volume 6 Number 5, June 2014
ISSN 2141-2162

Academic Journals

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
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,
Vellayani 695 522, Trivandrum, Kerala.

Dr.Saeed Aminzadeh
National Institute of Genetic Engineering and
Biotechnology (NIGEB)
Shahrak-e-Pajoohesh Km 15, Tehran-Karaj Highway,
Tehran, I.R.
Iran.

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, Maharashta,
India.

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

Dr. A. H. M. Mahbubur
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 *Nosch Labs*
Pvt Ltd Hyderabad,
India.

Noureddine Benkeblia
UWI - Department of Life Sciences
Mona Campus,
Kingston 7,
Jamaica.

Keutgen, Norbert
Uniwersytet Technologiczno-Przyrodniczy
im. Jana i Jedrzeja Sniadeckich w Bydgoszczy
Kadra Katedry Fizjologii Roslin
(Institute of Plant Physiology)
ul. Bernardynska 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, Profº 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.,
Melvisharam-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 Toyin Fasinmirin
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
UENF Av. José Carlos Pereira Pinto, 39. Pq. Vicente Dias. Campos RJ. Brazil.

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.

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

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

Gilberto Santos Andrade
Instituto 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 Ajasin 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
Haluoleo 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 Bakhoum
National Institute of Oceanography & Fisheries (NIOF), Egypt.

Antonio Americo Barbosa Viana
Embrapa Recursos Geneticos e Biotecnologia PBI-LPP1
PqEB Final W/5 Norte, Brasilia, DF – Brazil

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

Dr. Amaresh Chandra
Universidade Federal de Viçosa, Departamento de Biologia Animal, Programa de Pós-Graduação em Entomologia.
36570-000, Viçosa,
Minas Gerais State,
Brazil.

Dr. Atul Kumar
GB PANT University of Agriculture & Technology Department of Basic Science, College of Forestry & Hill Agriculture, HILL CAMPUS, PO Ranichauri, Tehri Garhwal, Uttarakhand State, India.

Prof. Levenko Boris
Natl. Botanical Gardens,NAS of Ukraine 01014 Kiev, 1 Timiryasevska st. Ukraine.

Dr. Dionisio G. Alvindia
Bureau of Postharvest Research and Extension CLSU Compound, Science City of Munoz, Nueva Ecija 3120, Philippines.

Dr. Bhoopander Giri
University of Delhi
Department of Botany, SSNC (University of Delhi) Alipur, Delhi 110036, India.

Dr. Anjuli Sood
University of Delhi
Department of Botany, University of Delhi, Delhi-110 007, INDIA.

Dr. A. K. Verma
G.B. Pant University of Agriculture & Technology, Pantnagar, Department of Biochemistry, College of
Basic Sciences,
India.

Dr. Anjana Jajoo
School of Life Science, Devi Ahilya University, Indore, DAVV
Khandwa Road campus, Indore 452 017, M.P., India.

Dr. Deepak Ganjewala
Vellore Institute of Technology University
55 Thennaraam Street, Vellore-632 014 (T.N.), India.

Dr. Geetha Govind
Max-Planck-Institute for Chemical Ecology
Hans-Knöll Straße 8, 07745 Jena, Germany.

Dr. Hossam El-Din Saad El-Beltagi
Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt, P.O.Box 12613 Egypt..

Prof. Dr. Md. Shahidul Haque
Dept. of Biochemistry and Molecular Biology University of Rajshahi, Rajshahi-6205, Bangladesh Bangladesh.

DR. P.K.NAGAR
Retired Senior Scientist, IHBT,Palampur, (H.P.), B.21/115-10A Batuk Dham Colony, Kamachha, Varanasi 221 010, INDIA.

Dr. Satyawati Sharma
Indian Institute of Technology
Centre for Rural Development & Technology,IIT Delhi-110016
Biomass Production on waste land, India.

Dr. Uğur Çakılcıoğlu
Fırat University Elazıg/TURKEY
Cumhuriyet M. Malatya C.
No:50/A.

Prof. Abdelrhani Elachqar
Faculty of Sciences Dhar El Mahraz, Fez, Morocco BP 1796, Fès-Atlas, Fès, Maroc, Morocco.

Ass. Prof. Jianfeng Xu
Arkansas State University
PO Box 639, State University, AR 72467 USA.

Ass.Prof. Jin Xu
Center for Agricultural Resources Research, Institute
of Genetics and Developmental Biology, Chinese Academy of Sciences
Huaizhong RD 286, Shijiazhuang, HeBei, China.

José Carlos Rebuglio Vellosa Ph.D
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

Dr. Krouma Abdelmajid
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

Dr. Majid Rostami
Malayer University
Department of Agriculture and Natural Resources, Postal code: 65719- 95863, University of Malayer Malayer, Iran.

Dr. Mohammad Nasir Khan
Aligarh Muslim University, Aligarh, INDIA Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh-202 002, U.P., India.

Prof. N.K.Matta
Kurukshetra University
Department of Botany, Kurukshetra University, Kurukshetra 136119, INDIA.

Dr. Naceur Djebali
Centre of Biotechnology Borj-Cedria (CBBC) BP 901, Hammam-Lif 2050 Tunisia.

Dr. Nader Chaparzadeh
Azarbaijan University of Tarbiat Moallem, Tabriz, Iran.

Nautiyal Prakash Chandra
Directorate Of Groundnut Research (Icar)
Post box, No. 5, Ivnagar Road, Junagadh-362001, Gujarat, India.

Prof. Hussein Fawzy Hussein Abouziena
National Research Center
Botany Department, National Research Center, Elbhoss Street, Dokki, Cairo, Egypt.

Dr. D.E. Chandrashekar Rao
National Research Council Canada / Plant Biotechnology Institute (NRC-PBI)
110 Gymnasium Place / Saskatoon, Saskatchewan S7N 0W9
Canada.

Dr. S.R Madhan Shankar
PSG College of Arts & Science
Civil Aerodrome Post, Coimbatore-641 014, India.

Prof. Dr. Safdar Hussain Shah
Institute of Biotechnology and Genetic Engineering NWFP, Agricultural University Peshawar, Pakistan.

Prof. Dr. Md. Shahidul Haque
Dept. of Biochemistry and Molecular Biology
University of Rajshahi, Rajshahi-6205, Bangladesh.

Dr. Sivakumar Swaminathan
Iowa State University (ISU)
G-319, Agronomy Department, ISU, Ames, Iowa - 50011, USA

Dr. Subrahmanyam Desiraju
Directorate of Rice Research (ICAR)
Plant Physiology Division, Rajendranagar, Hyderabad-500030, A.P. India.

Dr. Tariq Aziz Dr. Deepak Ganjewala
University of Agriculture, Faisalabad, Sub-Campus Depalpur, Dist. Okara, Pakistan.

Dr. Thangavel Palaniswamy *SUN*
YAT-SEN UNIVERSITY
GUANGZHOU, PR CHINA.

Yi-Ping Chen Ph.D
Institute of Earth Environment,
Chinese Academy of Science Fenghui S.R, 10, Xi'an Hi-Tech Zone, Xi'an, Chnia.

Saha Prasenjit
The Samuel Roberts Noble Foundation
2510 Sam Noble Parkway, Ardmore, Ok USA.

Abdul Khaliq Ph.D
Department of Agronomy
University of Agriculture
Faisalabad 38040, Pakistan.

Dr. Arafat Abdel Hamed abdel Latef
Assistant Professor of Plant physiology Botany Department
Faculty of Science at Qena
South Valley
University Egypt.

Dr. Ahmad Bybordi
Research Center of Agriculture and Natural Resources of East Azarbaijan
Member of Scientific Board of Research Center of Agriculture and Natural Resources of East Azarbaijan,
Tabriz. Iran.

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. India.

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. Rafiq 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
University of São Paulo,
Av. Pádua Dias, 11: CP 9. CEP 13418-900.

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, Serbi.

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

MS. C. Mehrnoush 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.

Dr. Diogo Pineda Rivelli
University of São Paulo,
Av. Prof. Lineu Prestes 580, São Paulo, SP, 05508-000.

Dr. Qiang Wang
Virginia Tech, 427
Latham Hall.

Dr. Foteini Hassiotou
University of Western Australia, 35
Stirling Highway, Crawley, WA
6009, Australia.

Dr. Nivedita Sahu
Indian Institute of Chemical
Technology, Chemical Biology
Laboratory
(NaturalProductChemistry), Uppal
Road, Hyderabad-500607.

Dr. Mohammad Anwar Hossain *Bangladesh*
Agricultural University, Assistant Professor,
Dept. of Genetics and Plant Breeding,
Bangladesh Agricultural University,
Mymensingh-2202, Bangladesh.

Dr. Ahmad Ali
National Institute of Pharmaceutical Education
& Research,
Dept of Biotechnology, NIPER, Jandaha Road,
Hajipur, Bihar, India, Pin – 844102, India.

Mr. Karthikkumar V
Annamalai University,
Department of Biochemistry & Biotechnology.

Dr.K.Rajendiran
Dept of plant science, Tagore Govt. college,
9, 4th cross, Tagore Nagar, Pondicherry – 605
008, India.

Dr. V. Balakrishnan *K.S.Rangasamy College of*
Technology, Department of Biotechnology,KSR Kalvi
nagar,Tiruchengode- 637215,Tamilnadu, India.

Dr. NourAli Sajedi
Department of Agronomy and plant Breeding,
Islamic Azad University, Arak Branch, Arak,
Iran.

(Dr) Ms. Rachel Predeepa
Not Applicable ,
2/387 Gokul Nagar, Kannanenthal Madurai.

Dr. Rajendra Gyawali
Department of Pharmacy and Biology, Kathmandu
University,
Dhulikhel,
Nepal.

Ms. Rocheli de Souza
UFRGS,
Porto Alegre,
Brazil.

Dr. Om Prakash Verma
Sam Higginbottom Institute of Agriculture,
Technology & Sciences (Formerly Allahabad
Agricultural Institute), Allahabad, U.P.,
Department of Molecular & Cellular Engineering,
Jacob School of Biotechnology & Bioengineering,
India.

Dr. Ashwani Kumar
JMIT, Radaur,
Department of Biotechnology, JMIT, Radaur-
135133, Haryana, India.

Dr. Sarfaraz F. A. Al-Bamarny
University of Duhok, College of Agriculture, Dept.
of Horticulture,
Duhok, Iraqi Kurdistan Region,
Iraq.

Prof. Wafaa Mohamed Shukry Abdel Meamem
Dammam University - Saudi Arabia,
Faculty of Science for Girl.
Biology Department,
P.O.Box: 838 Dammam 31113,
Saudi Arabia.

Dr. Stephka G. Chankova
Institute of Biodiversity and ecosystem Research,
BAS,
2 Gagarin str, 1113
Sofia, Bulgaria.

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 Chavan
K.r.c. College of horticulture, arabhavi 591 310, karnataka,
University of horticutlura sciences,
bagakot, India.

Dr. Maiti 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, Kita-gun, 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 Pacanoski
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, Hellbrunnenstraße 34, 5020, Salzburg, Austria.

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

Dr. Forouzandeh 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 VIIM, 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.

International Journal of Plant Physiology and Biochemistry

Table of Contents: Volume 6 Number 5 June, 2014

ARTICLES

academicJournals

Vol. 6(5), pp. 44-55, June 2014
DOI: 10.5897/IJPPB2014.0203
Article Number: 8A13CBB45432
ISSN 2006- 9871
Copyright © 2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/IJPPB

International Journal of Plant Physiology and Biochemistry

Full Length Research Paper

The physiological and behavioural responses of argan seedlings (*Argania spinosa* (L.) Skeels) to water stress in the semi-arid Western Algeria

Benaouf Zohra[1]*, Miloudi Ali[2] and Belkhodja Moulay[3]

[1]Laboratory of Ecology, Faculty of Biology, Houari Boumediene University of Sciences and Technology (U.S.T.H.B), El Alia, BP 32, Bab Ezzouar, 16111 Algiers, Algeria.
[2]Laboratory of Plant Biology, Faculty of Science, University of Mascara 29000, Algeria.
[3]Laboratory of Plant Physiology, Faculty of Science, University of Oran 31000, Algeria.

Received 1 March, 2014; Accepted 4 June, 2014

This paper presents the behavior, morpho-physiological and biochemical responses of argan seedlings to three water regimes at 30, 60 and 100% of field capacity. Based on the results of the growth parameters, we use the best result obtained from height growth. The increase in the number of leaves and thorns is obtained in seedlings subjected to water stress levels of about 60 and 100% against the application of a severe water stress of 30 and 0%. This led to reduction in the height of the stem, the number of leaves, and radial growth of biomass, and increased the length of the root portion. Regarding the water parameters, we noted a gradual decline in the relative water, the fresh and dry weight of leaves, stems and roots contents, with minimum values in the most severe stress (30 and 0%). Regarding the biochemical parameters, a decline in the relative water content is followed by a significant accumulation of soluble sugars and protein. Regarding the physiological parameters, stomata density intensifies level maintained at low water contents where the number of stomata per unit area behaves greatly compared to that of the hydrated treatments. Sweating is greatly reduced by increased water deficit; it is even more pronounced in the most severe treatment (30 and 0%).

Key words: *Argania spinosa*, germination, morphological, physiological, growth parameters, water, physiological water stress, biochemical water stress.

INTRODUCTION

The argan tree (*Argania spinosa* (L.) Skeels) is found only in large areas in Southern Morocco and the south-west of Algeria. It is the northernmost representative of a family in this region that hardly accepts tropical

*Corresponding author. E-mail: zbenaouf@yahoo.fr. Tel: +213776985892.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

Abbreviations: RWC, Relative water content; **DWR,** dry weight of the root; **DWA,** dry weight of the aerial part.

representatives (Lewalle, 1991). In Algeria, the argan tree is seen on the margins of Northwest Tindouf. It occupies the Wadi beds; tree density increases gradually toward the Atlantic Ocean, showing the tree of monotypic genus. It is the only survivor of tropical flora outside its natural range. It is considered an endemic tree (Otmani, 1995). The argan tree is considered as xerophytic and thermophilic species that adapts to high periods of prolonged drought and the drying effects of wind. This adaptation of the argan faculty is not related to the fact that this tree saves water, but its ability to draw water from great depths (Mokhtari, 2002). Also in the same periods of drought, the growth of certain branches of the tree decreases (El aboudi et al., 1991). According to Boudy (1950), more than a forest region is dry (arid and semi-arid floor), and the density of its older stock is reduced, because the roots need a large living space to draw soil water. The ecology of the argan tree is closely related to climatic factors; it is considered to be the least demanding tree species in rainfall (Boudy, 1952). Nevertheless, it needs a hydrometric degree air, where it can live only above a certain temperature in the moist coast (Victor, 1917). According to Nouaim and Chaussoud (1993), argan installs inward only weakly beyond 150 kilometer of the Atlantic Ocean, thus justifying that humidity seems to be a key parameter for this ecology species. In this context, Wattier (1917) and Emberger (1939) and Boudy (1950) argue that the altitudinal limit of the argan tree is the isotherm (m = 3.8°C) and a remarkably high thermal support of the order of 50°C. Similarly, Emberger (1924) reported that cold is the determinant of the geographical distribution of the argan tree. Plants generally require optimal environmental conditions for normal growth, but they are often subject to extreme factors of water potential, temperature and salinity, generating different types of stress (Hopkins, 1999; Bouaouina et al., 2000). However, water stress has a special place because of its frequency, and the place that water plays in metabolic processes (Turner, 1990; Bálo et al., 1995). Due to its role in photosynthesis, transport and accumulation, as well as multiplication and cell enlargement, water has a critical role in the growth and development of plants (Rascio et al., 1990; Blake et al., 1991; Medrano et al., 1992; Cabeza et al., 1995). Damage caused by water stress results from drying protoplasmic; the departure of water causes an increase in the concentration of solutes. If the volume of protoplasm decreases, membrane integrity and protein is also affected by the drying, this causes metabolic disorder (Hopkins, 1999). Water deficit can also reduce turgor pressure of the plant. This loss of turgor state may cause the cessation of cell elongation in particular (Gate, 1995). The aim of this work is to determine argan seedlings' morpho- physiological parameters for assessing the degree of tolerance of the species to drought conditions (water stress). This section focuses on water parameters (fresh and dry weight of leaves, stems, roots, and the relative water content), physiological parameters (stomata density and transpiration), and biochemical parameters through an analysis of foliar soluble sugars and protein.

MATERIALS AND METHODS

Argan seedlings (nr = 200) were obtained after germination tests were performed in the laboratory. The seedlings were taken to a greenhouse at the University of Mascara and controlled under light and temperature conditions. The night and daytime temperatures are maintained respectively (15 and 28°C) and photoperiod is 12 h day. Seedlings are irrigated regularly every 10 days and divided into 4 groups:

Lot 1: Witness (T) was maintained at 100% of field capacity.
Lot 2: Seedlings were maintained at 0% of field capacity.
Lot 3: Seedlings were maintained at 60% of field capacity.
Lot 4: Seedlings were maintained at 30% of field capacity.

To determine the field capacity of the pot, we weighed pots containing 5 kg of dry substrate used in the experiment (W1 = 5 kg of dry soil). Then we proceeded to irrigate seedlings to saturation, while covering the pots with black plastic to prevent evaporation of water. Thereafter, we soaked the pots for 48 h. Finally, we reweighed the W2 pots (weight saturation). So, the difference between W1 and W2 represents the amount of water that the soil was able to keep at 100% of field capacity. On the other hand, due to the evolution of the biomass of seedlings over time, the amount of water returned to each pot and irrigation frequency was adjusted every 10 days for 6 months (Figure 1).

Measured parameters

Water parameters

1. Measurement of fresh and dry weight of leaves, stems, and roots. The dry root (DWR) Weight: It is obtained by passing in an oven at a temperature of 60°C for 48 h (Belkhodja and Sotani, 1992). Air dry weight: The dry weight of the aerial part (DWA) of stems and leaves is evaluated by passing in the oven at a temperature of 60°C for 48 h.
2. Measurements of parameters (DWR and DWA) involved the ratio: DWR/DWA (dry weight of the root/dry weight of the aerial part).
3. Measurement of relative water content (RWC).

Thus, several parameters are used to quantify the water status of plants subjected to water deficit. We include the percentage of moisture, relative water content, and the percentage of imbibitions. So, despite the difficulties inherent to the operations of weighing the plant studied and changes in fresh and dry weight, we chose to study the relative water content. It expresses the amount of water present in % of the saturated amount measured and allows a physiological assessment of water status of the plant as well as the water potential. The formula below shows the estimate of this parameter:

$$RWC = (FW - DW). 100 / (W Sat - DW)$$

FW = Fresh weight; W Sat = weight of saturation; DW = dry weight
1. The fresh weight is determined by weighing the sheet immediately after collection.

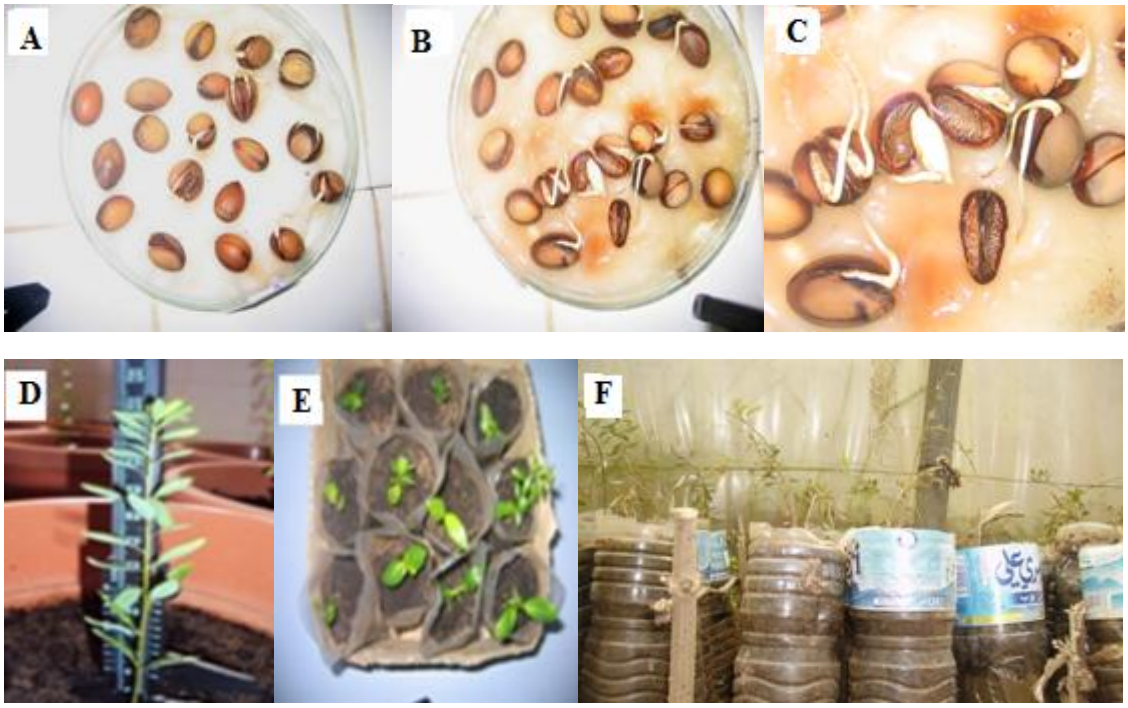

Figure 1. A, B and C. Germination tests in laboratoty; D. Argan seedling ; E. Acclimatization of arganseedlings ; F. seedlings argan in a greenhouse.

2. The weight of water saturation is obtained by placing the sheet distilled water for 24 h.
3. The dry weight is obtained by placing the sheet in the oven at 80°C for 24 h.

Physiological parameters

Measurement of stomata density

Stomatal density is obtained by the method of Dohman et al. (1991) and used by Lemeu (1999 and 2000). Principle: The leaf prints are seen by spreading a few drops of nail polish on both epidermis (upper and lower) of the sheet. After drying, the footprints are taken by a transparent adhesive tape, which is adhered to a blade in order to count the number of stomata (mm^2) by using microscope equipped with ocular micrometer calibrated and supporting a phototube ZEISS brand. Finally, measurements are made on the number of stomata per unit area on the two types of leaf epidermis.

Measurement of transpiration

The middle of the leaf sample (perspiration is maximum) is weighed (fresh weight of the leaf = P1). The same sample is used to calculate the leaf surface; whereas in our case, the sheet is treated as an ellipse. The following formula allows us to calculate the leaf surface (Boudersa, 1998),

S = 3.14. (A. b / 4)

With: - a: This is the length of the leaf

- B: This is the maximum width of the leaf
Then the sheet is left in the open air for one (01) h, and then the weight loss after water is evaluated (Wb).

Therefore, the calculation of sweating is achieved by the following formula:

Swt = (Wa - Wb). S-2. T -1.

Swt: Sweating (mg H_2O. area $^{-2}$. time $^{-1}$); Wa: fresh leaf weight; Wb: weight loss after water; S -2: unit leaf area (cm^2); T -1: Unit of time (second) s.

Biochemistry parameters

Protein determination

The method used is that of Troll and Lindsley (1955), simplified and developed by Dreier and Goring (1974). Principle: It is to take 100 mg of plant material (1/3 middle of the sheet), and then add 2 ml of 40% methanol. The whole was heated to 85°C in a water bath for 60 min. After cooling, the following extracts were added:

1. 1 ml of acetic acid (CH_3COOH).
2. 25 mg of ninhydrin ($C_6H_6O_4$).
3. 1 ml of a mixture containing 120 ml of distilled water, 300 ml of acetic acid, and 80 ml of phosphoric acid (H_3PO_4, d = 1.7).

The mixture is boiled for 30 min, then the solution turns red. After cooling, 5 ml of toluene was added to the solution and then shaken, at two separate phases (upper and lower phases). After removal of the lower phase, the upper phase is recovered and dried by the addition of a spatula of sodium sulfate anhydrous Na_2SO_4. Finally, we determine the optical density (528 nm). The values are converted to protein content from the standard curve, whose relationship is as follows:

Y = 0.1043 x.

Figure 2. Determination of soluble sugars.

Determination of soluble sugars

The total soluble sugars (sucrose, glucose, fructose, their methyl derivatives and polysaccharides) are determined by the method of Dubois et al., 1956. Principle: It is to take 100 mg of plant material (1/3 of the middle sheet) in test tubes, add 3 ml of 80% ethanol to extract sugars and then leave in room temperature for 48 hours. At the time of the assay, the tubes are placed in an oven at 80 ° C to evaporate the alcohol in each tube; 20 ml of distilled extract (sample solution) water is added. In clean glass tubes, we put 2 ml of the sample solution, 1 ml of 5% phenol (phenol is dissolved in distilled water), and adds up quickly 5 ml of 96% concentrated sulfuric acid (d = 1.86), while avoiding pouring acid on the tube walls. We obtained an orange yellow solution on the surface; it passes through a vortex to homogenize the color of the solution. The tubes were left for 10 min and placed in water for 10 to 20 min at a temperature of 30°C.
Note: The color of the reaction is stable for several hours. The values obtained are reported in the standard range, Y = 4.3918 x - 0.1946 (Figure 2).

Statistical analysis

All tests were repeated three times on measures of water, biochemical and morpho- physiological parameters. The results presented as histograms usually mean values. They were produced by Excel. In the case of paintings, the results were subjected to statistical analysis (analysis of variance, ANOVA / MANOVA) with the help of STATISTICA software.

RESULTS

Observations and measurements of water physiological and biochemical parameters

Because the physiological measurements are made at the end of the period of water stress for different treatments, we perform statistical analysis of morphological, physiological and biochemical parameters under the effect of water stress at the same time (after 9 months).

Water Parameters

Effect of water stress on fresh and dry weight of leaves, stems, and roots

The decrease in fresh and dry weight of leaves, stems and roots is due to the level of water stress.–Indeed, in the argan seedlings, processed at 0% and 30% of the

Table 1. The standard deviation and the average fresh weight of leaves, stems, and roots of argan seedlings.

Stress (%)	The average		
	leaves	stems	roots
0	0.37 ±0.09	0.75 ±0.15	1.20 ±0.14
30	0.84 ±0.11	1.58 ±0.16	2.35 ±0.14
60	1.19 ±0.14	2.07 ±0.14	3.10 ±0.20
Witnesses (100%)	1.36 ±0.58	2.77 ±0.19	3.75 ±0.12

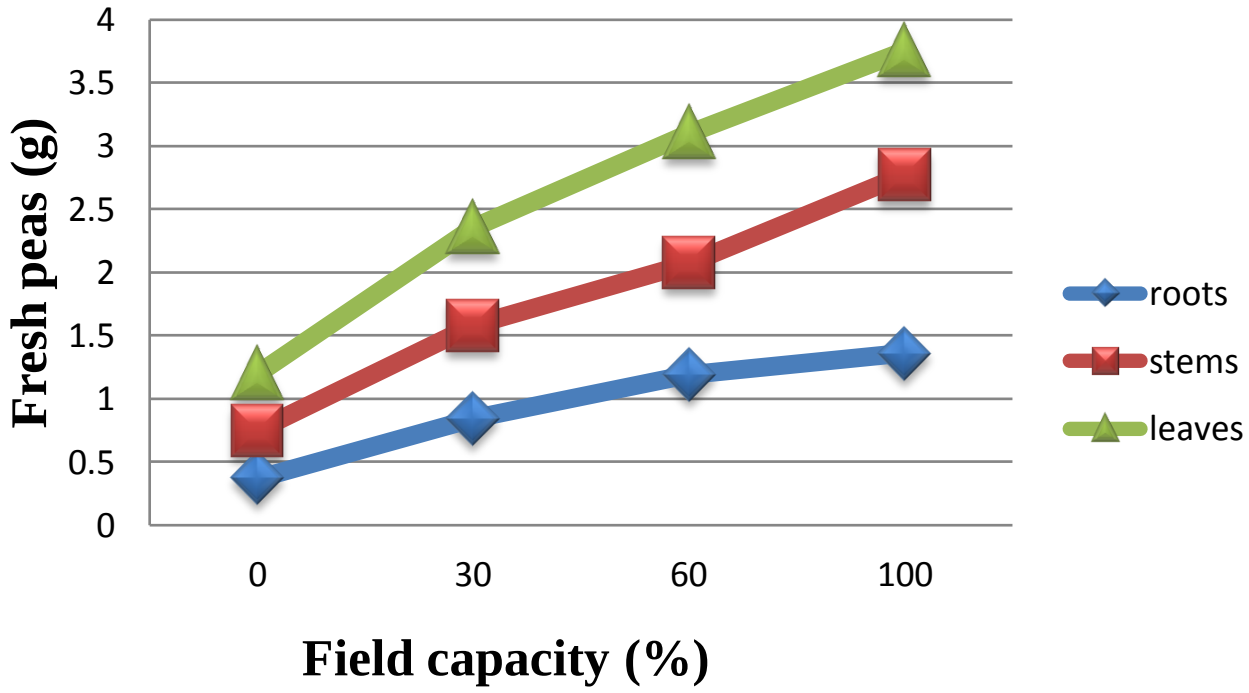

Figure 3 . Change in fresh weight of leaves, stems, and roots of seedlings argan, aged 9 months and subject to different levels of water stress. Base of formula

field capacity, we observed lower values of fresh weight of leaves, stems, and roots, respectively in the following order: 0%: 0.37 g, 0.75 g and 1.2 g; 30%: 0.84 g, 1.58 g and 2.35 g (Table 1, Figure 3).

The maximum fresh weight of leaves, stems, and roots was found in seedlings treated with 60% and 100% of field capacity, in the following order: 60%: 1.19 g, 2.07 g and 3.1 g; 100 %: 1.36 g, 2.77 g and 3.75 g. Concerning the dry weight, leaves, stems and roots of argan and control seedlings treated at 30% of field capacity had low values, in the following order: 0.125, 0.290 and 0.27 , and 0.19, 0.58 and 0.59 g. And the highest values are observed in seedlings treated at 60 and 100% of field capacity, in the order of 0.275, 0.7 and 1.18, and 0.39, 0.87 and 2.18 g. The results show that the aerial part is more sensitive to the effect of water stress than the root. The reduction in vegetative growth observed in seedlings can be explained by the increased osmotic pressure of the medium, which prevents the absorption of water by the root system and consequently causes a reduction in the growth of the vegetative apparatus. Similar effects have been seen on the vegetative growth in the seedlings of argan (Table 2, Figure 4).

Effect of water stress on the DWR/ADW ratio

It is reported that root dry weight and air dry weight depend mainly on water supply. The DWR/ADW ratio has changed by 1.55, 2.52, 4.07 and 4.6 in argan seedlings witness at 30%, 60% and 100% of field capacity. Lack of water caused a decrease in the DWR / ADW ratio between the two groups; at the same time the seedling root system is well developed and the air dry weight values are still low in relation to the dry weight root.

Indeed, the lowest values of DWR and ADW are

Table 2. The standard deviation and the average dry weight of leaves, stems, and roots of argan seedlings.

Stress (%)	Average		
	leaves	stems	roots
0	0.125 ±0.026	0.29 ±0.099	0.27 ±0.067
30	0.190 ±0.039	0.58 ±0.122	0.59 ±0.137
60	0.275 ±0.075	0.70 ±0.081	1.18 ±0.252
Witnesses (100%)	0.39 ±0.196	0.87 ±0.200	2.18 ±0.122

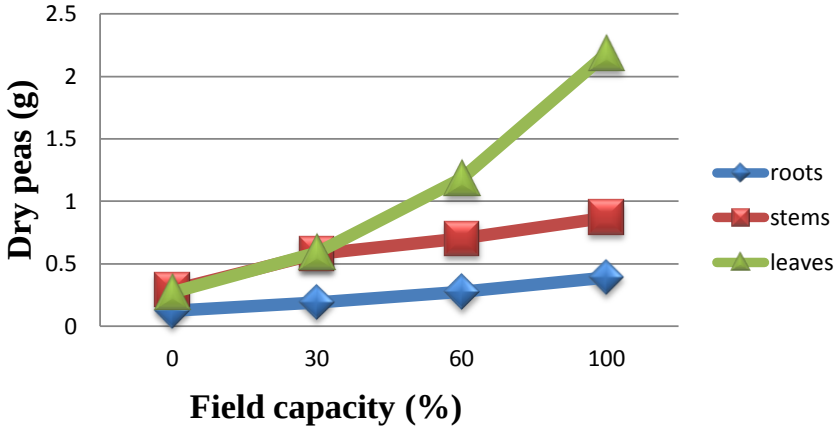

Figure 4. Change in dry weight of leaves, stems, and roots of seedlings argan, aged 9 months and subject to different levels of water stress.

Table 3. The standard deviation and the mean DWR / ADW ratio argan seedlings.

Stress (%)	The average
0	1.55 ±0.11
30	2.52 ±0.071
60	4.07 ±0.17
Witnesses (100%)	4.60 ±0.25

Table 4. The standard deviation and the mean of the relative water content of argan seedlings.

Stress (%)	The average (%)
0 %	59.89 ±8.17
30 %	75.55 ±6.90
60 %	87.60 ±3.29
Witnesses (100%)	95.45 ±1.51

recorded in the control seedlings with respective values of 0.35 and 0.24 g. While the mean values of the DWR and ADW are recorded in seedlings at 30 and 60% of field capacity, with 0.86 and 1.55 and 0.34 and 0.39 g values, respectively. The maximum values of DWR and ADW are identified in seedlings at 100% of field capacity, with respective values of 2.24 and 0.47 g. The results show that the ratio of dry root weight and dry weight of air is negatively correlated with the intensity of water stress applied. This correlation is driven by the values of the DWR/ADW which remain lower in the most stressed argan seedlings (Table 3, Figure 5).

Effect of water stress on the variation of the relative water content (RWC)

The analysis of the results shows that the relative water content is greatly influenced by the water regime. The relative water content decreases as the intensity of the stress is high. The highest in water tenure is noted in the lot with 60% of field capacity. It has a maximum value of about 100% (control) of field capacity, ranging between 87.60 and 95.45%.

The relative lower water tenure is stored at 0% of field capacity, with 59.89% value. The average value is found in the batch of 30% of capacity field, in the order of 75.55%. The analysis of the relative water content (RWC) describes in a comprehensive manner the water status in response to water stress (Table 4, Figure 6).

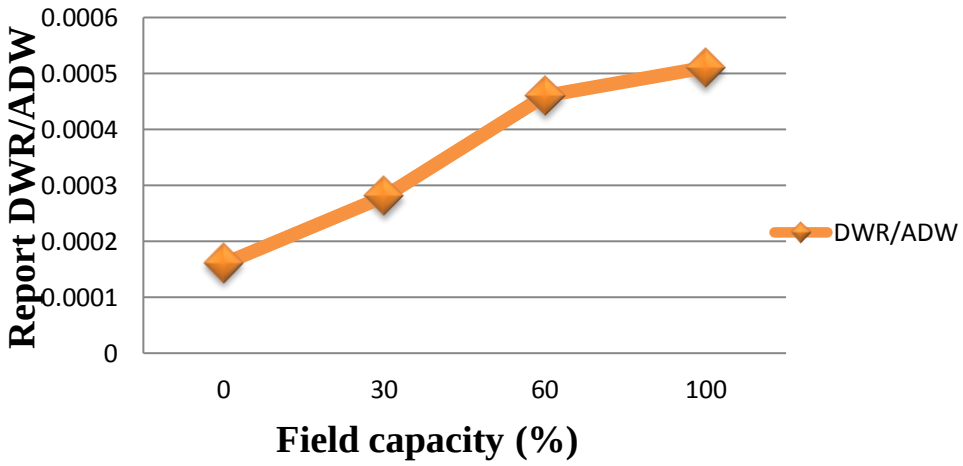

Figure 5. Change DWR / ADW ratio argan seedlings aged 9 months and subject to different levels of water stress.

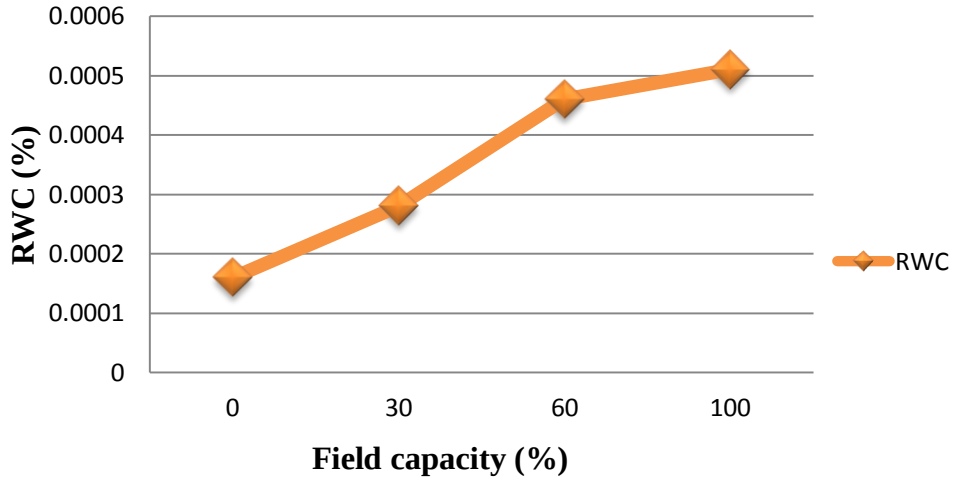

Figure 6. Variation of relative water content of the argan tree seedlings, aged 9 months and subject to different levels of water stress.

Physiological parameters

Stomata density

The density of stomata in the upper and lower epidermis is strongly influenced by the water regime. Thus, we find that increased water deficit causes an increase in stomata density at both upper and lower leaf surfaces of the argan tree (Table 5).

In seedlings of the argan tree with 0% of field capacity, stomata density of the upper and lower epidermis has almost doubled (14.8 s/mm2, 24.5 s/mm2). In seedlings treated with 30 and 60% of field capacity, stomata density of the upper and lower epidermis increased slightly at 12.8 s/mm2, 18.8 s/s/mm2, and 10 s/s/mm2, 15.1 s/mm2. And lower stomata density on the upper and lower epidermis is recorded in seedlings treated with 100% of field capacity, at 5.7 s/mm^2, 11.7 s/mm^2 (Figure 7 and 8).

Effect of water stress on the variation of sweating

The intensity of sweating is conditioned with the level of water stress. Moreover, we find that the lowest level of perspiration is recorded in seedlings treated with 0% of field capacity and those treated with 30% of field capacity, in the order of 0.000164 mg H2O. S^{-1}.cm^{-2} and 0.000285 mg H2O. S^{-1}.cm^{-2}.

On the other hand, maximum sweat is obtained in seedlings treated with 60 and 100% of field capacity, with values of 0.000463 mg H_2O.S^{-1}.cm^{-2} and 0.000517 mg

Table 5. The standard deviation and the mean stomatal density argan seedlings.

Stress (%)	The average (s/mm^2)	
	lower Epidermis	upper epidermis
0	14.8 ±1.39	24.5 ±2.67
30	12.8 ±1.03	18.8 ±1.68
60	10 ±1.33	15.1 ±1.68
Witnesses (100%)	5.7 ±2.00	11.7 ±1.56

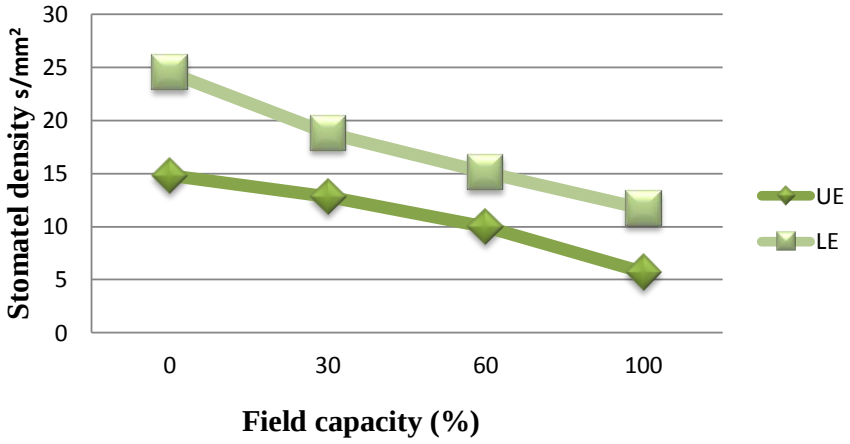

Figure 7. Variation in stomata density of seedlings argan, aged 9 months and subject to different levels of water stress.

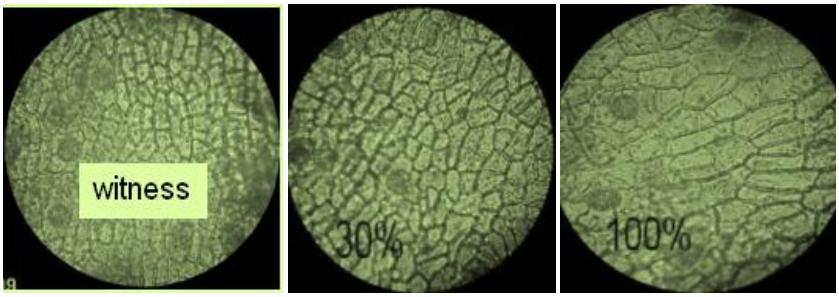

Figure 8. stomatal density (stomata per unit area) of the upper epidermis of the leaves of the argan tree, conducted under different water regimes.

Table 6. The standard deviation and the mean transpiration argan seedlings.

Stress (%)	The average (mg H_2O. S^{-1}.cm^{-2})
0	0.00016 ±0.000034
30	0.00028 ±0.000018
60	0.00046 ±0.000020
Witnesses (100%)	0.00051 ±0.000021

$H_2O.S^{-1}.cm^{-2}$. The relationship between water deficit and physiological parameters shows the need for maintaining good sweat (Table 6, Figure 9).

Biochemical parameters

Effect of water stress on the variation in the content of proline

In the results, we note that water stress is causing an increase in proline tenure in leaves of argan seedlings.

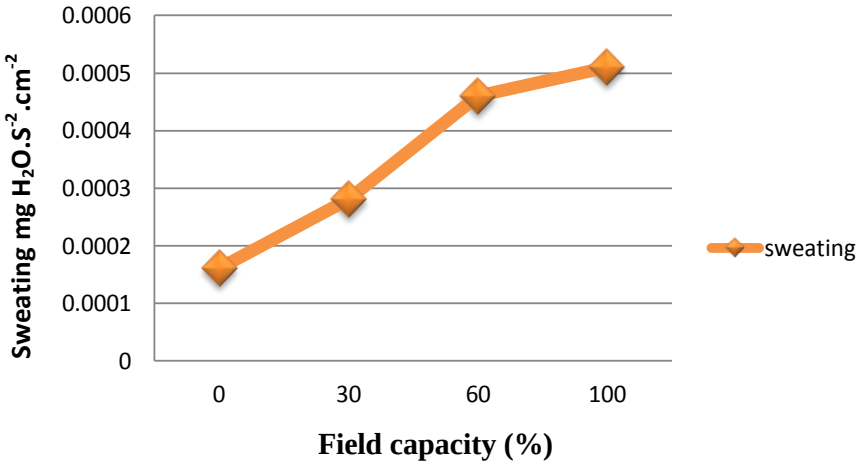

Figure 9. Change sweating argan seedlings, aged 9 months and subject to different levels of water stress.

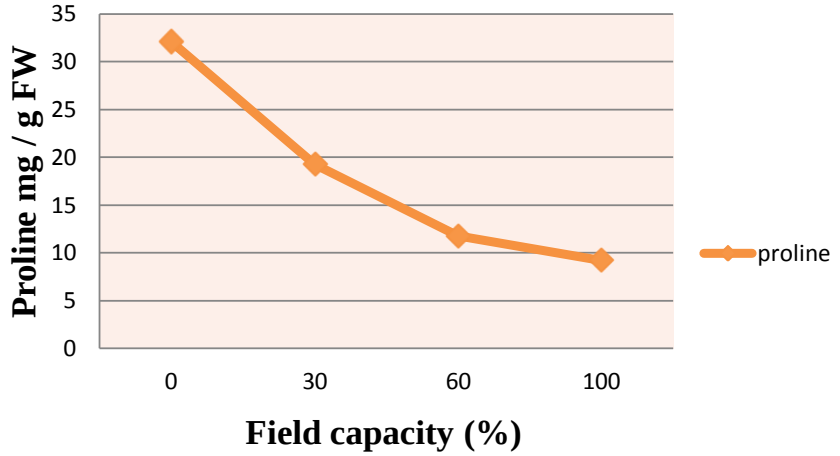

Figure 10. Change in proline accumulation in leaves of argan tree seedlings, aged 9 months and subject to different levels of water stress.

Table 7. The standard deviation and the average proline argan seedlings.

Stress (%)	The average (mg/g PF)
0	0.05 ±0.0013
30	0.037 ±0.0022
60	0.025 ±0.0016
Witnesses (100%)	0.013 ±0.0012

Indeed, in control seedlings and those treated with 30% of field capacity, the contents of proline are more marked, in the order of 0.0505 mg/g FW and 0.0379 mg/g FW.

The average values of proline levels are identified in argan seedlings treated at 60% of the field capacity, of about 0.0251 mg/g FW. And the lowest levels of proline are stored in the control seedlings treated with 100% of the field capacity of about 0.0132 mg/g FW (Table 7, Figure 10).

Effect of water stress on the accumulation of soluble sugars

The levels of applied water stress cause significant increase in the levels of soluble sugar. Indeed, when the level of water stress becomes severe, the argan tree seedlings record very high levels of soluble sugar. So the highest level of soluble sugar is stored in the argan tree seedlings with 0% of field capacity and those treated with 30% of field capacity, with values of 32.28 mg/g FW and

Table 8. The standard deviation and the mean of soluble sugars argan seedlings.

Stress (%)	The average (mg/g PF)
0	32.12 ±0.49
30	19.27 ±0.337
60	11.74 ±0.26
Witnesses (100%)	9.21 ±0.43

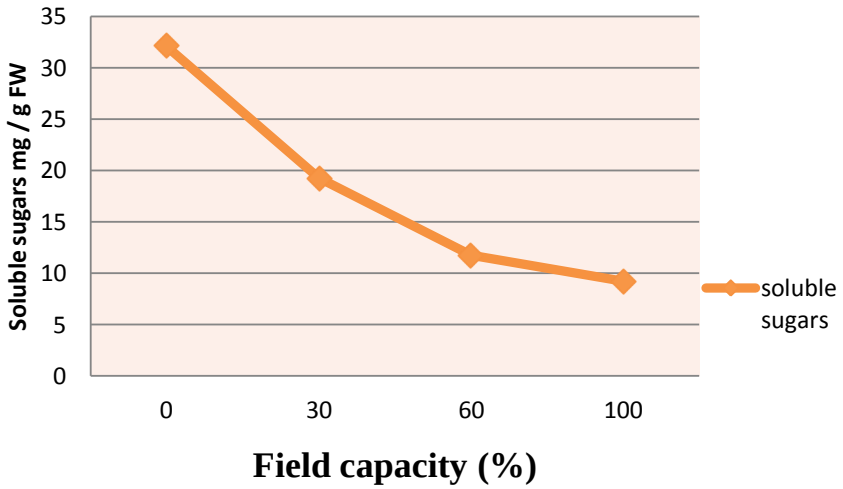

Figure 11. rate of accumulation of soluble sugars in the leaves of the argan tree seedlings, aged 9 months and subject to different levels of water stress.

19.34 mg/g FW. The contents of soluble sugar readings are the lowest in seedlings treated with 100% of field capacity (9.348 mg/g FW).In seedlings treated with 50% of field capacity, we find that the levels of soluble sugar are average, about 11.60 mg/g FW. The effect of water deficit on biochemical parameters is used to highlight a highly significant effect of water deficit on the accumulation of leaf protein (Table 8, Figure 11).

DISCUSSION

The effect of drought is also expressed by a progressive and rapid slowdown in growth as the primary water deficit reduces turgor and consequently the expansive power of the leaves. Results linking water deficit and water parameters show that the effect of water stress on root growth, leaf and stem is very remarkable, corresponding to a statistically significant difference noted between the different treatments. It is also shown that the aerial part is more sensitive to the effect of water stress than the root. The reduction of vegetative growth observed in seedlings argan can be explained by the increase of the osmotic pressure of the medium, which prevents the absorption of water by the root system and thus leads to a reduction in the growth of the vegetative apparatus. Similar effects have been seen on the vegetative growth in seedlings of argan. The same results were also obtained by the same species (Harrouni, 1995); and according to Thakur and Rai (1982), water deficit causes a delay in plant growth. It results in a reduction in the height and diameter of the nod, a shortening of the internodes and a decrease in the number of leaves and leaf surface (Aspinall, 1986). With a decrease in root growth depending on the degree of exploitation of the soil by the root system, the argan seedlings tend to reduce the length of the roots to overcome the effect of water stress caused. The results show that the ratio of dry root weight and dry weight of air is negatively correlated with the intensity of water stress applied. This correlation is driven by the values of the PSR / PSA which are still lower in the most stressed seedlings of argan. This indicates that the water deficit did not affect similarly the two parts of the plant. The increase in the ratio could be explained by an increase in root growth mostly affected by lack of water than air. The preferential development of the root system to the detriment of the aviation system is considered by many authors as a criterion for drought resistance; making better use of available water more accessible (Bensalem, 1993; Bchini et al., 2002). The analysis of the relative

water content (TRE) is used to describe in a comprehensive way the water status in response to water stress and to assess the ability to achieve a good osmoregulation and maintain cell turgor (Eljaafari, 2000).

The water status of the plant, expressed by the relative water content was sensitive to changes in water available in the soil. Indeed, water deficit causes a regression of the values of relative water content in which these findings are confirmed by the net negative and significant relationship revealed between water deficit and relative water content. During our experiments, the results obtained indicate that the application of severe water deficit negatively affects and significantly reduces the relative water content of the tissue. The greater the intensity of water stress, the more the water content is lowered in the most severe water stress (0 and 30%). This reduction is due to dehydration, leading to a loss of water in the cells. The relative water content is high compared to control seedlings subjected to water stress levels at 60 and 100%. This is probably due to active osmoregulation. Similar work was done by Morgan (1984) Bennaceur (1994) and Nouri (2002) on different types of plants subjected to water stress of different intensities.

The relationship between water deficit and physiological parameters shows the need for maintaining good sweat, and consequently maintaining stomatal coverage during water deficit directly involved in the level of the relative water content (Denden and Leneur, 1999; Nouri, 2002). This indirectly conditions the use of water effectively for photosynthesis (Erchidi et al., 2000). According to Slama et al. (2005), the reduction of water loss by stomatal closure is a means by which plants adapt to drought. Thus, our results show a strong positive relationship between water stress and stomatal density at both upper and lower epidermis of the leaves. According to Slama (2002), the increase in the number of stomata per unit area could be a factor in resistance to water stress if it is accompanied by a good physiological activity.

The increase in stomatal density can increase the net CO_2 assimilation and reduce water loss. Indeed, a large number of stomata can cause stomata small size and fast closing (Slama et al., 2005). Passive cell dehydration of argan seedlings induces turgor loss at the cellular level. To overcome this, plants try to limit water loss, which results in decreased sweating due to the closure of the stomata (Yakhlef, 2001; Nouri, 2002). The results show a very highly significant positive correlation between transpiration and relative water content. Estimated through water loss by excised leaf transpiration is greatly reduced by the accentuation of the water deficit. This decrease was more marked in the most severe treatment. Similarly, these data are confirmed through the work of Clark et al. (1989, 1991), which indicates that sweating is a key parameter variation of the water status of the plant and affects productivity. The effect of water deficit on biochemical parameters is used to highlight a highly significant effect of water deficit on the accumulation of leaf proline. Similar results were encountered in different forest species such as *Fraxinus excelsior* and *Quercus petrea* (Ladefoged, 1963), *Quescus coccifera* (Losch et al., 1982) and *Argania spinosa* (Peltier et al., 1992). However, Claussen (2005), working on tomato under salt and drought stress, suggested that the accumulation of proline could be due to an induction/activation of the enzyme involved in the biosynthesis of proline or lowering of glutamate in its oxidation and improved proteins. One of the causes of the accumulation of proline is membrane proteolysis which could accumulate proline following a disturbance in the metabolism of proteins (Bezzala, 2005). Clifford et al. (1998), in their work, show an accumulation of proline and soluble sugars (hexoses , glucose). This is in agreement with our results that a very highly significant positive correlation exists between soluble sugars and proline. This suggests the latter is extremely sensitive to the synthesis of proline at the reduction of NADP + and enhances cellular data, stating that the synthesis of proline is a means for regulating cellular redox potential "contribution in energy adjustment" (Bellinger et al., 1987; Rai, 2002). Furthermore, it can be inferred that soluble sugars (sucrose and glucose) are effectors of the accumulation of proline. Mastrangelo et al. (2000) and Nouri (2002) suggest that the ability to accumulate in these genotypes osmoticums (sugars and proline) is used as the basis for selecting drought tolerant genotypes. The levels of soluble sugars sheets show a very highly significant negative correlation between the relative water content and accumulation of soluble sugars (glucose). Therefore, an increase in soluble sugars was seen in many woody plants such as Eucalyptus and microtheca (Chunyang, 1998; Pesoli et al., 2003). According to these authors, the increase in soluble sugars is attributed to degradation of starch due to the rapid conversion of sucrose and inhibition of the synthesis of starch reserves.

REFERENCES

Aspinall D (1986). Effects of water and salinity stress in relation to expansion of the leaf surface. Oust J. Plant Physiol. 13:59-73.

Bálo B, Varga I, Váradi G, Misik S (1995). Drought damage in vineyards. INRA, Inter drought, VIII-27, (Transferring drought tolerance from tetraploid fescue to ryegrass), in European vineyards. Eur. J. Entomol. 96:375-380.

Bchini H, Dalloul A, Sayar R (2002). Genetic variability of some parameters of wheat root system due (*triticum durum* Desf) under two water regimes. Edit. Plant Génétic Resources Newsletter. 192:25-31.

Belkhodja M, Soltani N **(**1992). Responses of the bean Vicia faba L salinity Study of germination of some lines to growth. Determined. Bull. Soc. Bot. FR. 357-368.

Ben naceur M (1994). Contribution to the assessment of the degree of resistance to water stress (drought and excess water) in barley (Hordeum vulgare L.) and fescue (Festuca arundinacea Schreb). State doctoral thesis. INA (el Harrach in Algeria). 1-13.

Bensalem M (1993). Comparative study of drought adaptation in wheat,

barley and triticale. Dan: Drought tolerance of cereals in Mediterranean area. Genetic diversity and varietal improvement. The symposia LNRA. 64:275-298.

Bezzala A (2005). Introductory essay to the Argan (Argania spinosa (L.) Skeels) in the area M'doukel and evaluation of some parameters of drought resistance. Magister Thesis, University of Batna, Algeria. 68.

Blake TJ, Bevilacqua ZJJ (1991). Effects of repeated stress on turgor pressure and cell elasticity exchange in black spruce seedlings. Can. J. For. Res. 21:1329-1333.

Bouaouina S, Zid E Hajji M (2000). Tolerance to salinity, chlorophyll fluorescence and ion transport in durum wheat (Triticum turgidum L.). Option Mediterranean. 239-243.

Boudy P (1950). North African Forest Economy (monographs and treatment of tree species). VolumeII (1), Larose. 382-416.

Boudy P (1952). Guide forest in North Africa, Rustic House Edition (Paris).

Cabeza C, Kin A, Ledent JF (1995). Effect of irrigation and tiller removal on development and yield of the main shoot in two wheat cultivars. INRA. Inter. drought. VIII. 29.

Gate PH (1995). Ecophysiology wheat. And technical documentation. ISBN. Ed Tec and Doc. Lavoisier, Paris. 429.

Chevalier (1943). Sapotaceae arid regions. J. Inst. Appl. Bot. Trop. Agric. 260-262 and 153-159.

Chunyang L (1998). Some aspects of leaf water relations in four provenances of Eucalyptus michrotheca seedlings. Forest Ecology and Management 111. 3036-308.

ClarkE JM, Romagosa I (1991). Evaluation of excised-leaf water loss rate for selection of durum wheat for dry environments. Physiology-breeding of winter cereals for stressed Mediterranean Environments (Montp. Franc, 3-6 July. 1989). Edit. INRA. Col. 55:411-413.

Claussen W (2005). Protein as a measure of stress in tomato plants. Plant Science. J. Plant Nutr. Soil Sci. 168 (2. 168, 2416, 248.

Clifford SC, Karnd T S, Corlett JE, Jochi S, Sankhla N, Popp M, Hamlyn GJ (1998). The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in ZiZiphus mauritiana (Lamk.). J. Exp. Bot. Vol. 49(323):967-977.

Denden M, Leneur R (1999). Measurement of transpiration by the Penman-monteith model. Drought. John Libbey Eurotext. Medical and Scientific Publications France. 10:39-44.

El aboudi A (1990). Typology of sub-Mediterranean and ecophysiology argan argan tree (Argania spinosa (L.) Skells) in Sub (Morocco) Thesis Univ. Grenoble I. 113.

El jaafari S (2000). Durum wheat breeding for abiotic stresses resistance: Defining physiological traits and criteria. Option méditerranéenne. 40:251-256.

Emberger L (1924). About the geographic distribution of the argan tree. Bull. Soc. Sci. Nat. Morocco. 4(7):151-153.

Emberger L (1939). Overview on the vegetation of Morocco. Commentary phytosociological map of Morocco to 1/500000, Veroff. Geobot. Inst. Rübel in Zürich (14) and Mem. Sc Nat. Morocco. I.S.C., Rabat. 40-157.

Erchidi AE, Benbella M, Talouizte A (2000). Relationship between certain parameters controlling water loss and grain yield of new varieties of durum wheat subjected to water stress. Mediterranean option. 40.279-282.

Harrouni MC, Zahri S, El hamaid A (1995).Transplant seedlings Argan: combined effect of cultivation techniques and water stress. Conference proceedings intrnational forest to desertification "case of argan" Faculty, Agadir. 26, 27 et 28 octobre. 115-33.

Hopkins (1999). Introduction to plant physiology. Second edition. The University of Western Ontario. Edit. John Wilay and sounds. Inc. 512.

Lewalle J (1991). Argan exceptional tree. Royal Air Morocco magazine. 53:12-14.

Lösch R, Tenhunen JD, Pereira JS, Lange OL (1982). Dinrnal courses of stomata resistance and transpiration of wild and cultivated Mediterranean perennials at the end of the summer dry season in Portugal. Flora. 172:138-160.

Mastrangelo AM, Rascio A, Mazzucco l, Russo M, Cattivelli l, Di fonzo N (2000). Molecular aspects of abiotic stress resistance in durum wheat. Option méditerranéenne. 40:207-213.

Medrano H, Aguilo F socias FX (1992). Effect of drought on subterranean clover: 1 - leaf water relationships of plants Subjected to rapid or slowly induced drought. Photosynthetica. 413-419.

Miloudi A (2006). The physiological and biochemical responses of the argan (Argania spinosa L.Skeels_natural abiotic factors. Doctorate thesis, University of Oran ES SENIA). 1-2:30-90.

Mokhtari M (2002). Quick Production plants argan Apt transplantation. Laboratory of Plant Ecophysiology; Agronomic and Veterinary Institute Hassan II. Agadir. Bull. Information and Liaison PNTTA No 95. 1-3.

Nouaim R, Chaussod (1993) .Argan (Argania spinosa (L) Skeels). The flamboyant newsletter network members' tropical trees 27. Septembre1993. 50-64.

Nouri L (2002). Osmotic adjustment and maintenance of photosynthetic activity in durum wheat (Triticum durum, Desf.), under conditions of water deficit. Magister thesis in Plant Biology.INA (el Harrach). 4-16.

Otmani N (1995). The problem of the argan tree. In: Proceedings of study days on the argan tree (Marrakech, 29-30 September 1995). 3.

Peltier JP, El aboudi A, Carlier G, Doche B (1992). Water potential and stomata conductance of argan leaves (Argania spinosa (L.) Skeels) at the beginning and during the dry season in the Souss (Western Morocco). Bull. Ecol. 5-16.

Pesoli P, Gratani L, Larcher W (2003). Responses of Quercus ilex from different provenances to experimentally imposed water stress. Biologia plantarum. 577-581.

Rai V K (2002). Role of amino acids in plant responses to stresses. Biologia Plantarum. 481- 487.

Rascio A, Cedola MC Toponi M, Flagella, Zwittmer G (1990). Leaf morphology and water status in exchange Triticum durum under stress. Physiologia plantarum. 78:462-467.

Slama A, Salem M, Ben naceur M, Zid E (2005). Cereals in Tunisia production, effect of drought resistance and mécaniseme edit. Drought. 16:225-229.

Thakur PS, Rai VK (1986). Effect of water stress on protein content in two maize cultivars differing in drought resistance. Biologia Plant (Praha) 1982. 24:96-100.

Turner IB (1990). The extent and pattern of osmotic ajustement in white clover (Trifolium repens L.) during the development of water stress. Ann. Bot. 66:721-727.

Victor P (1917). Morocco geography. Story development. Edi. Colin. 63.

Ykhlef N (2001). Photosynthesis, photochemical activity and tolerance to water deficit in durum wheat (Triticum durum Desf.). Doctorate Thesis in plant physiology.

Wattier R (1917). Note on the argan tree of Morocco. International Colonial Exposition. Paris 1931. 57p. University of Sidi-bel-Abbes. 120.

academicJournals

Vol. 6(5), pp. 56-65, June 2014
DOI: 10.5897/IJPPB2014.0207
Article Number: AC9F57E45433
ISSN 2006- 9871
Copyright © 2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/IJPPB

International Journal of Plant Physiology and Biochemistry

Full Length Research Paper

Comparative changes in metabolism of *Vigna radiata* by foliar and root application of brassinolide at different concentrations

Sana Riaz, Neelofer Hamid and Afshan Rahman*

Department of Botany, University of Karachi, Karachi 75270, Pakistan.

Received 30 April, 2014; Accepted 3 June, 2014

The present study shows the effect of the foliar and root application of brassinolide on growth and metabolism of *Vigna radiata*. For this purpose, three concentrations of brassinolide (1, 0.01 and 0.0001 ppm) were applied on the plant. Brassinolide (BL) solutions were applied as foliar and root treatments for 21 days after sowing (DAS). Samples were collected after two and four weeks of treatments. Content of total sugars, reducing sugars, chlorophyll and proteins, activity of peroxidase (EC 1.11.1x), leaf area and height of plants were estimated. All these parameters increased significantly by 1 ppm foliar spray of BL as compared to the other concentrations. It was concluded that BL enhanced growth of *V. radiata*. L. under both root and foliar applications, but foliar treatment was more effective.

Key words: *Vigna radiata,* foliar spray, root irrigation, brassinolide, concentrations.

INTRODUCTION

Vigna radiata L. belongs to the pulse family. It is a rich source of protein, especially in under developed countries like Pakistan where proteinaceous diet is not accessible to every person. Its worth is very important. The total area under major pulse crops in Pakistan is about 1.5 m hectares. With the development of short duration and uniform maturing varieties, mung bean can be fitted in various cropping systems (Pakistan Agriculture Research Council).

Brassinolide is a compound of Brassinosteroids (BR) class, the sixth class of plant hormones, discovered in the 1970s. Brassinosteroids are involved in many physiological processes like stem elongation, xylem differentiation, root inhibition, pollen tube growth, ethylene biosynthesis, leaf epinasty, regulation of gene expression and photosynthesis (Sasse, 2003). A lot of work has done on the effects of this hormone on plants grown under different stress conditions and it is found to be effective in amelioration of stress. BRs can act efficiently in plants as immunomodulators when applied at the appropriate concentration and at the correct stage of plants' development (Bajguz and Shamsul, 2009). The foliar spray either with 24-epiBL or 28-homoBL signi-ficantly enhanced the growth, photosynthesis, and protein content in mung bean; they play a critical role in a range of developmental processes, for example stem and root

*Corresponding author. E-mail: afshan.rahman48@yahoo.com.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

growth, floral initiation, and the development of flowers and fruits (Shamsul and Ahmed, 2003). Kalinich et al. (1985) investigated *Phaseolus vulgaris* and *Phaseolus aureus* and elucidated that BRs had an impact on the transcription, leading to an increase in protein content. Exogenous application of BRs enhanced the prospective efficiency of crops by stimulating cell elongation, vascular differentiation and/or proton pump (Hayat and Ahmad, 2003).

In the present study, the effects of brassinolide on growth and metabolism of *Vigna radiata* L. were studied. Observation regarding total proteins, total carbohydrates, reducing sugars, photosynthetic pigments, peroxidase activity, and leaf area and plant heights were recorded.

MATERIALS AND METHODS

Field experiments were performed. For this purpose, healthy seeds were sterilized with 1% solution of mercuric chloride for 5 min and then washed with distilled water several times before sowing. Plastic pots of 6 inches diameter were used for the experiment. In each pot, equal quantity of soil was used. Brassinolide solution with concentrations of 1, 0.01 and 0.0001 ppm was applied to plants 21 days after sowing (DAS) at two leaf stage (as foliar and root treatments to separate set of plants and to compare results of both treatments). For each treatment, three replicates were used. For biochemical evaluations, samples were taken at 2nd and 4th week after treatment. Leaf area and height of plants were taken when plants reached their maximum heights.

Estimation of chlorophyll

Chlorophyll was estimated by the method of Maclachaln and Zalik 1963. 0.5 g of fresh leaves was taken and macerated in 7 ml of 80% acetone and centrifuged at 1000 rpm for 5 min. The debris was then washed 3 times using 3 ml of 80 % acetone each time. The supernatants were then pooled and made up to the final volume with 80 % acetone. Optical density of this solution was then recorded at 663 and 645 nm.

The chlorophyll content was calculated using the formula given below and expressed in milligram per gram fresh weight.

$$\text{Chlorophyll a (mg / g)} = \frac{12.3\ D663 - 0.861\ D645 \times V}{D \times 1000 \times W}$$

$$\text{Chlorophyll b (mg /g)} = \frac{19.3\ D645 - 3.6\ D663 \times V}{D \times 1000 \times W}$$

Total carbohydrates

0.3 g fresh leaves were taken and macerated in 7 ml of tris HCl buffer and then centrifuged at 2500 rpm for 20 min. Supernatant was collected for estimation. 1 ml of aliquots was taken and 4 ml distilled water was added to it. 10 ml anthrone reagent was used. The reaction mixture was incubated at boiling water bath for 16 min, and then cooled. Optical density was recorded at 680nm. For reagent blank, 1 ml buffer was used instead of leaf extract. Sucrose (1000 µg/ml) was used to prepare standard curve. Total carbohydrates were determined by the method of Hassid and Abraham (1957).

Estimation of reducing sugars

Determination of reducing sugars was done according to Bernfeld (1955). Leaf extracts were prepared by taking 0.3 g fresh leaves, macerated in 7 ml of tris HCl buffer and centrifuged at 2500 rpm for 20 min. 1 ml of this leaf extract was mixed with 2ml of DNS (3,5-dinitrosalicylic acid). It was prepared by dissolving 1g of DNS in 50ml water and then 30 g of potassium sodium tartarate was added slowly. Then, 20 ml 2N NaOH was added and diluted up to 100ml. Samples were incubated in boiling water bath for 15 min. Reaction was terminated in an ice bath and optical density was recorded at 540 nm. Maltose was used to prepare calibration curve in µg/ml.

Bradford protein assay

Protein standards

Protein standards were prepared in the same buffer as the samples to be assayed. A convenient standard curve was made using bovine serum albumin (BSA).

Assay reagent

Coomassie blue G250 (100 mg) is dissolved in 50 ml of methanol and filtered through a glass-fiber filter. The solution is added to 100 ml of 85% H_3PO_4 and diluted to 200 ml with water. The assay reagent is then diluted 4 folds.

Assay

0.04 ml of aliquots was added to 2 ml of assay reagent. After 30 min of incubation at room temperature, the optical density was recorded at 595 nm. The total protein was calculated in µg/ml by using standard curve.

Peroxidase activity

Peroxidase activity was analyzed by the method of Chance and Maehly (1955). 0.5 g sample was crushed in 5 ml of phosphate buffer of pH = 6. It was centrifuged at 1600 rpm for 10 min and then the supernatant used for the estimation of enzyme was collected. 0.1 ml enzyme extract of plant was mixed with 2.1 ml deionized water, 0.32 ml buffer and 0.16 ml hydrogen peroxide (5%) (freshly prepared). 0.32 ml pyrogallol solution (5 %) (freshly prepared) was added and the reaction mixture was chilled. The absorbance of the reaction maximum was measured at 420 nm with a double bean UV/ visible spectrophotometer. For reagent blank, buffer was used instead of enzyme extract. The calibration curve was prepared by using peroxidase solution. Enzyme activity was measured in µg/min/mg FW by the following formula:

$$\text{Enzyme activity} = \frac{\text{Reading from std. curve x Amount of extract}}{\text{Activity time x wt. of material x volume of extract used in test}}$$

RESULTS AND DISCUSSION

The aim of this study was to check whether brassinolide foliar spray or root irrigation is beneficial for plant growth and metabolism and to find which method and

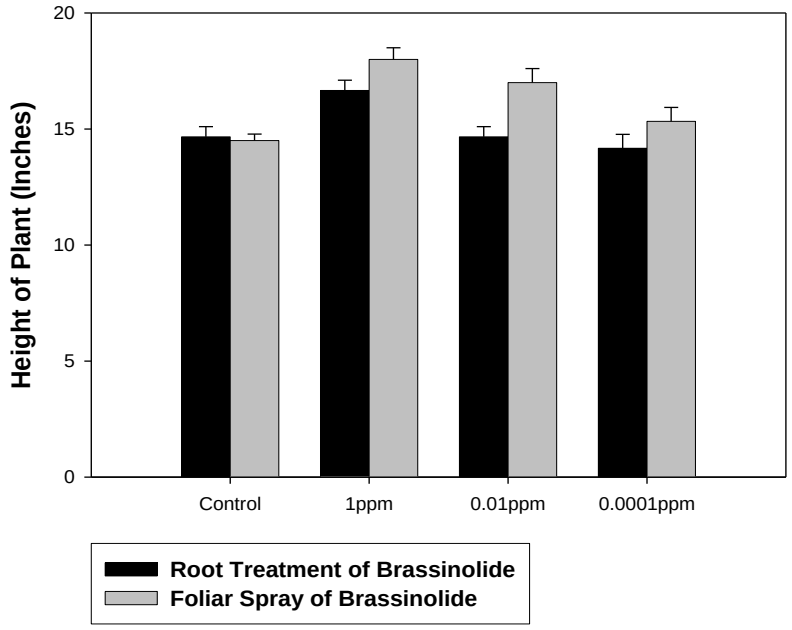

Figure 1. Effect of different concentrations of brassinolide on height (inches) of *Vigna radiate.* Bars show standard error, SE ± (n=3).

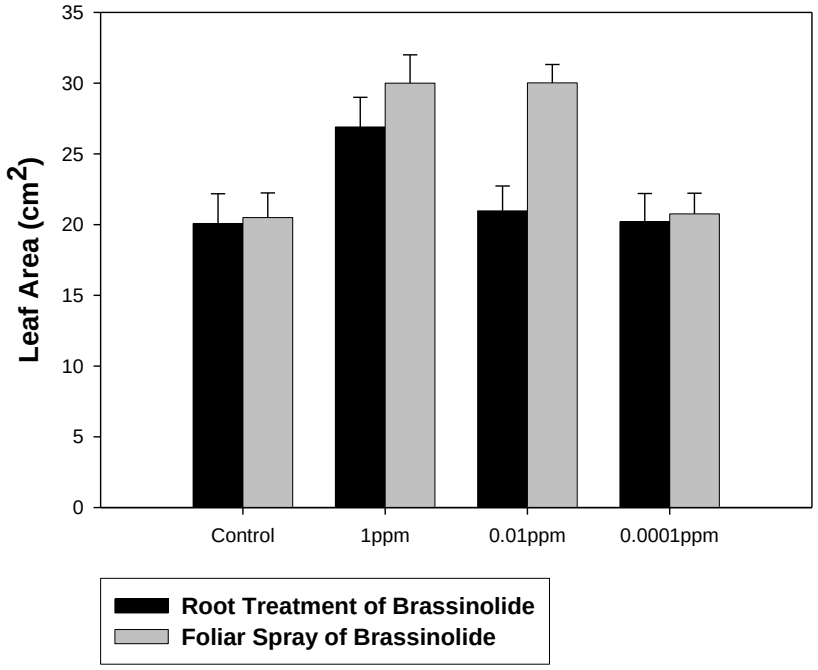

Figure 2. Effect of different concentrations of brassinolide on leaf area (cm^2) of *Vigna radiata*. Error Bars represent standard error of mean (n=3).

concentration of brassinolide application enhance the quality of crops under normal conditions.

The morphological parameters like plant height, leaf area increased significantly ($P < 0.05$) with sprays and dosage of brassinolide compared to control and root irrigation (Figures 1 and 2). Similar result was reported by Ramraj et al. (1997). Plants sprayed with brassinolide reached the reproductive stage earlier than plants which

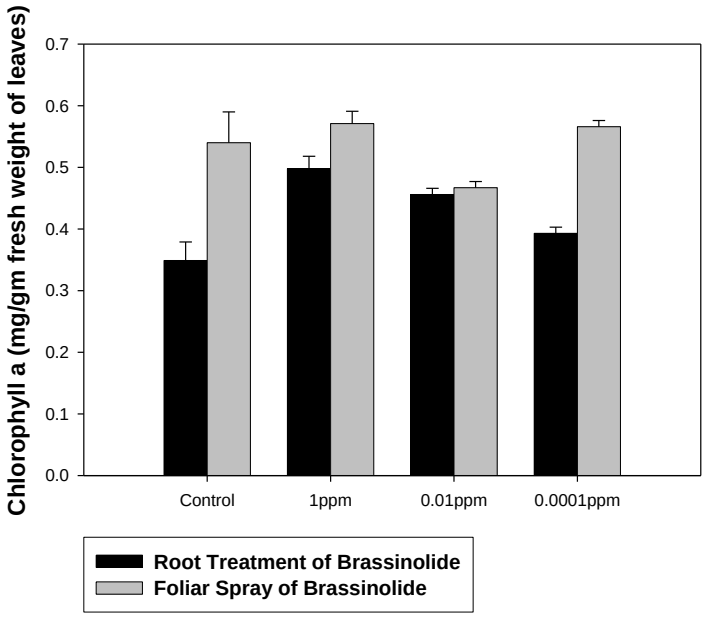

Figure 3. Changes in chlorophyll 'a' in *Vigna radiata* by foliar and root application brassinolide at different concentrations after 2nd week. Significantly different at ($p < 0.05$).

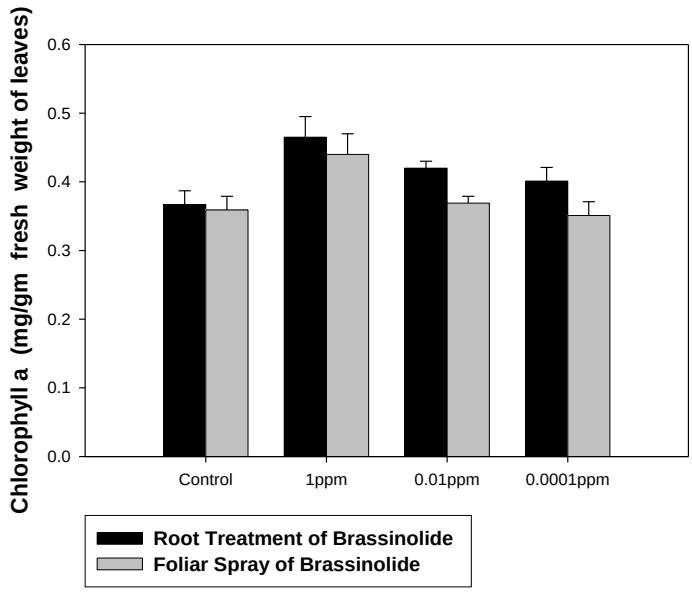

Figure 4. Changes in chlorophyll 'a' in *Vigna radiata* by foliar and root application brassinolide at different concentrations after 4th week. Significantly different at ($p < 0.05$).

were not sprayed with brassinolide. This increase in height and leaf area is due to the increase in cell division and cell elongation. This is supported by Rao et al. (2002).

Brassinolide affects the growth and quality of crop when treated with irrigation water or spray method. The amount of chlorophyll "a" and chlorophyll "b" increased significantly when plants were sprayed with solutions of BL (Figures 3 and 4). Chlorophyll content decreased with decrease in concentration (1ppm, 0.01ppm, 0.0001ppm)

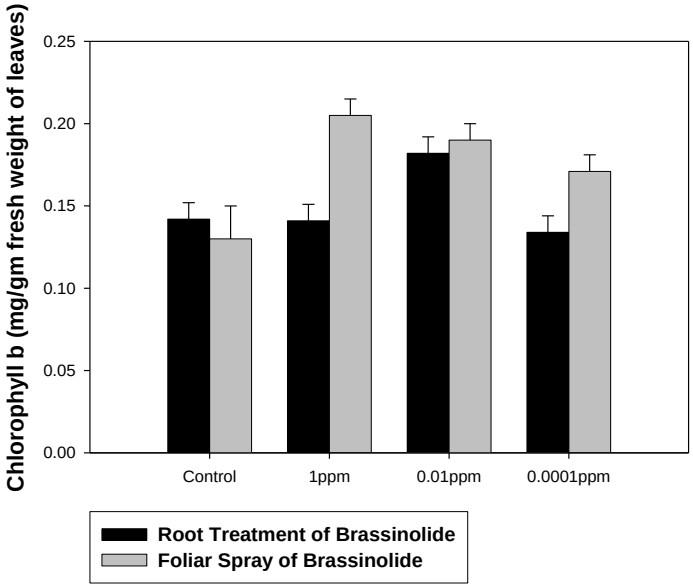

Figure 5. Changes in chlorophyll 'b' in *Vigna radiata* by foliar and root application brassinolide at different concentrations after 2nd week. Significantly different at (p< 0.05).

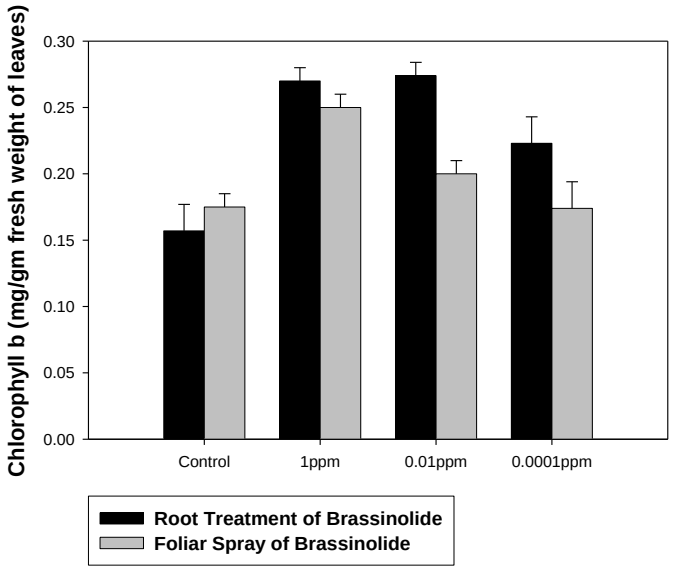

Figure 6. Changes in chlorophyll 'b' in *V. radiata* by foliar and root application brassinolide at different concentrations after 4th week. Significantly different at (p< 0.05).

but remained higher than control. These results were supported by the work of Sairam (1994). The interaction of BL and time period also showed significant (P< 0.05) results. In 2nd week, the amount of chlorophyll pigments was increased with foliar spray but later, after the 4th week, root application was effective (Figures 5 and 6).

Foliar spray of BL increases significantly (p<0.05) the amount of total sugars and reducing sugars as compared to root application after 2nd week of treatment (Figures 7 and 8). It was also revealed that the effect of BL increases with the time duration as the amount of total sugars is high in the samples taken at 4th week (Figures 9

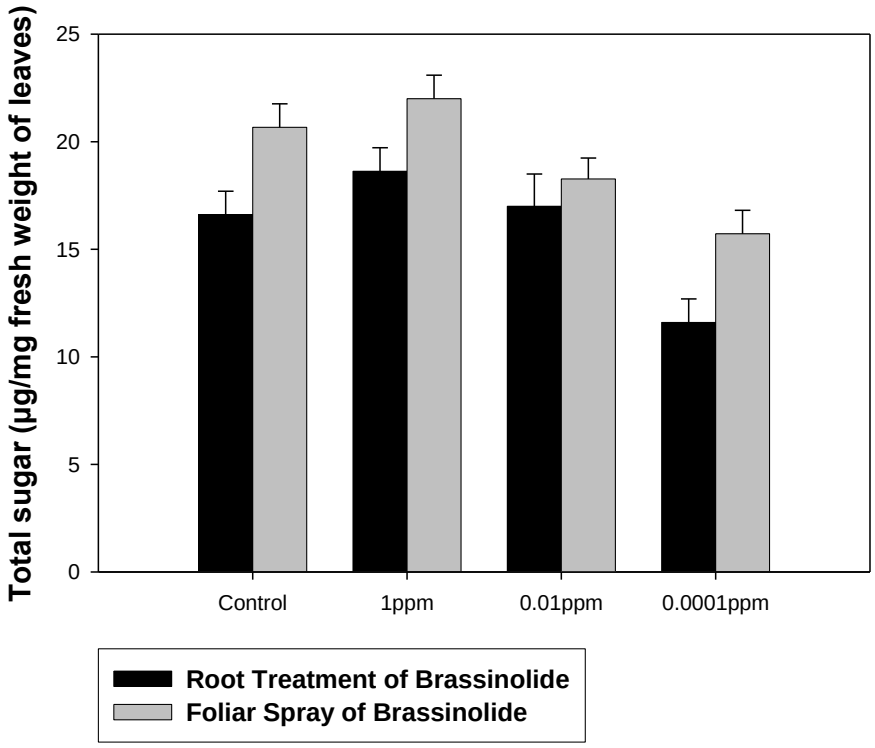

Figure 7. Effect on the amount of total sugar in *Vigna radiata* treated after 2nd week at different concentartions of brassinolide apply as foliar and root treatment.

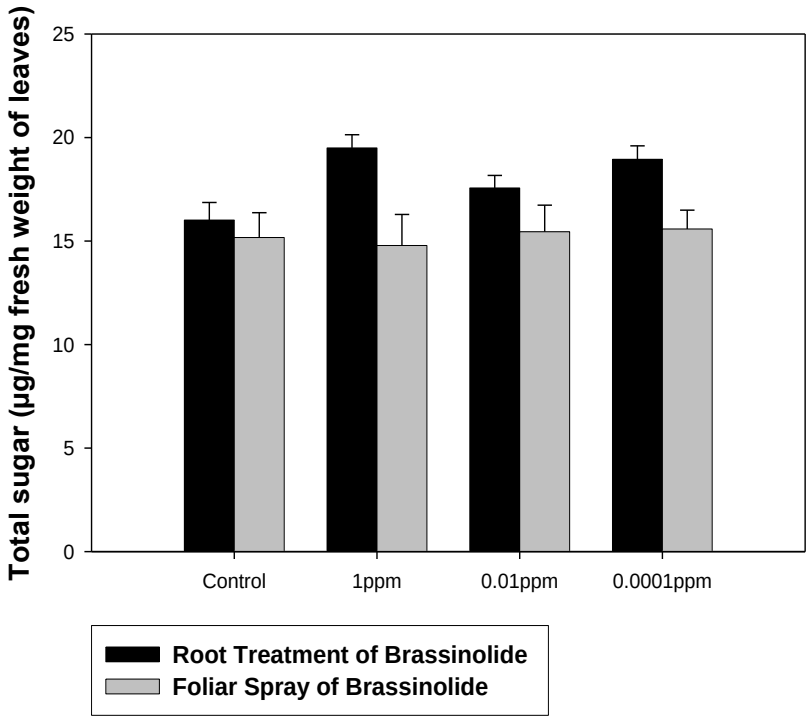

Figure 8. Effect on the amount of total sugar in *Vigna radiata* treated after 4th week at different concentartions of brassinolide apply as foliar and root treatment.

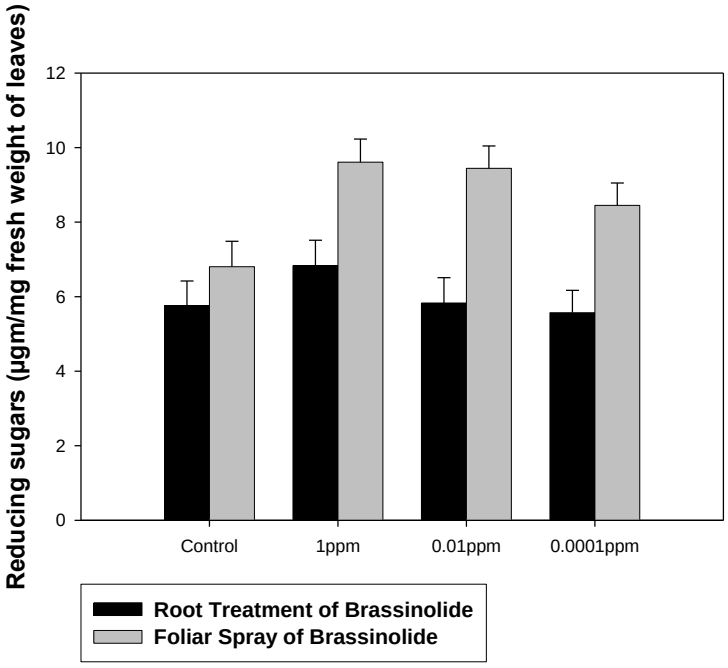

Figure 9. Effect of different concentrations of brassinolide on amount of reducing sugar in *Vigna radiata* after 2nd week of treatment. Significant results at ($p < 0.05$).

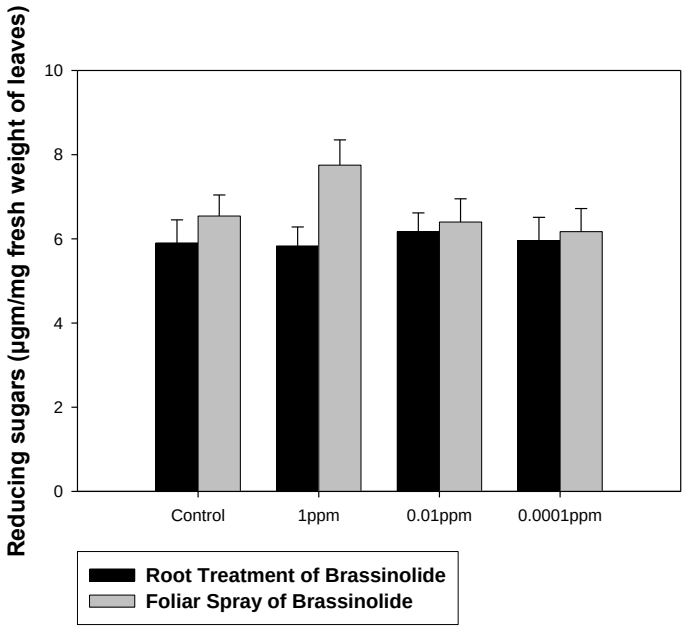

Figure 10. Effect of different concentrations of brassinolide on amount of reducing sugar in *Vigna radiata* after 4th week of treatment. Significant results at ($p < 0.05$).

and 10). The highest amount of total sugars was found in plants sprayed with 1 ppm of BL. This indicates that brassinolide increases the metabolic activities in mung beans (Vardhini and Rao, 1998). The increase in total

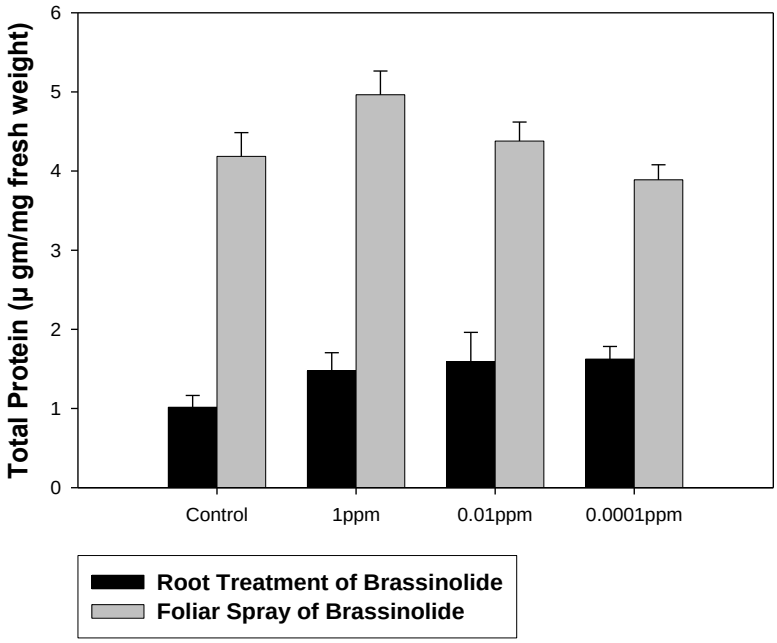

Figure 11. Changes in total protein (µg/mg fresh weight) in *Vigna radiata* by foliar and root application of brassinolide after 2nd week.

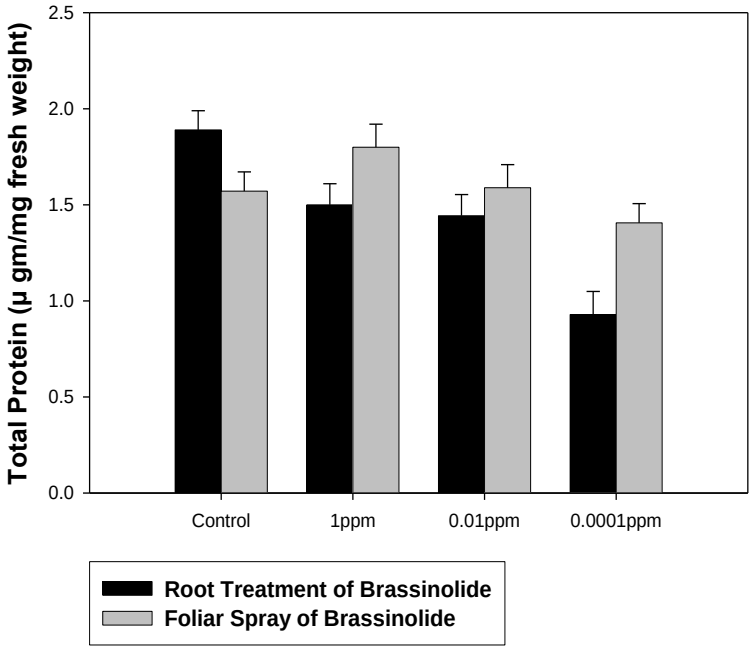

Figure 12. Changes in total protein (µg/mg fresh weight) in *Vigna radiata* by foliar and root application of brassinolide after 4th week.

sugar was noticed due to the rise in the rate of photosynthesis with increase in the amount of chlorophyll content (Braun and Wild, 1984).

Total proteins were significantly enhanced ($P< 0.05$) by spraying different concentrations of BLs solutions compared to root application (Figures 11 and 12). Result

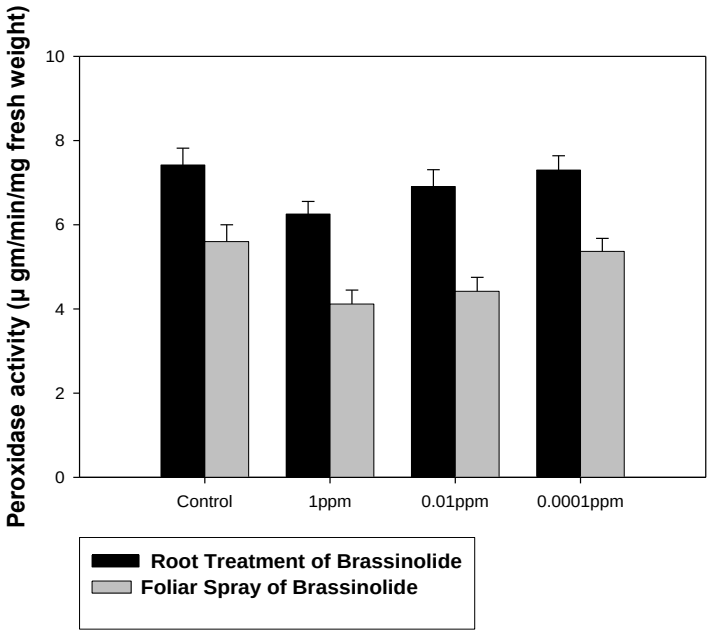

Figure 13. Peroxidase activity (µg/min/mg fresh weight) show changes in *Vigna radiata* after 2nd week of brassinolide application. Bars represent standard error of mean (n=3).

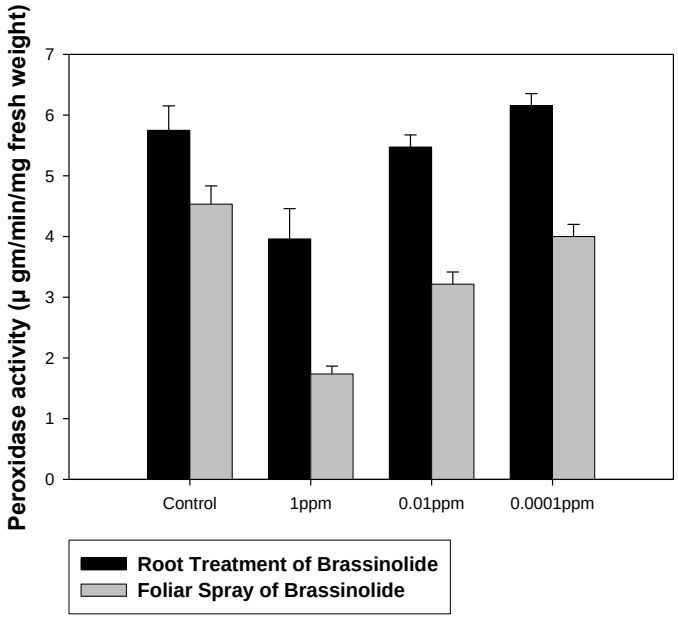

Figure 14. Peroxidase activity (µg/min/mg fresh weight) show changes in *Vigna radiata* after 4th week of brassinolide application. Bars represent standard error of mean (n=3).

analysis showed the direct relationship between amount of proteins and concentration of BL. Similar results were observed by many researchers and co-workers (Sharma et al., 2014; Bajguz, 2000).

The activity of peroxidase was decreased by the supplementation of BRs rather than sprays and root irrigation. It was higher in control samples (Figures 13 and 14). Result of peroxidase activity is supported by

Arora et al., 2008. Higher peroxidase activities are closely associated with growth of the plants (Zheng and van Huystee, 1992). BL suppressed the activity of peroxidase. It indicates advanced and healthier growth of the plant over the control sample because it was revealed that activity of peroxidase is responsible for the gluconeogenesis of lipids (Jones, 1972).

Conclusion

Our investigation demonstrated that both foliar and root application of brassinolide are effective. Initially, in 2nd week, effect of foliar treatment is significant because it is readily absorb in plant cell through stomata and enhanced metabolic activities. But as time passes, plants suck up the brassinolide by their roots and utilize it in their cells. This plays a constructive role in their growth and metabolism. The order of effective response to BL treatment was 1 > 0.01 > 0.0001 ppm > control.

REFERENCES

Abbasi FM, Ahmad H, Perveen F, Inamullah, Sajid M, Brar DS (2010). Assesment of genomic relationship between *Oryza sativa* and *Oryza australiensi*s. Afr. J. Biotechnol. 9(12):1312-1316.

Arora N, Bhardwaj R, Sharma P, Arora HK. (2008). 28-Homobrassinolide alleviates oxidative stress in salt-treated maize (*Zea* mays L.) Plants Braz. J. Plant Physiol. 20(2):153-157.

Bajguz A (2000). Effect of brassinosteroids on nucleic acids and protein content in cultured cells of Chlorella vulgaris. Plant Physiol. Biochem. 1(38):209-215.

Bajguz A, Shamsul H (2009). Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiol. Biochem. 47:1-8

Bernfeld P (1955). Amylase, alpha and beta. In: Colwick, S.P., Kaplan, N.O. (Eds), Methods in enzymology, Academia Press, Newyork. 1:149-158.

Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72(1-2):248-254.

Braun P, Wild A (1984). The influence of brassinosteroid on growth and parameters of photosynthesis of wheat and mustard plants. J. Plant Physiol. 116:189-196.

Chance B, Maehly AC (1955). Methods in enzymology. II:773-775.

Hassid WZ, Abraham S (1975). Chemical procedures for the analysis of polysaccharides. Determination of glycogen starch. Determination of glycogen with anthrone reagent. In: Methods in enzymology (Eds) S.P. and N.O. Kaplan Academic press, New York. 35-36.

Hayat S, Ahmad A (2003). Soaking seeds of Lens culinaris with 28-homobrassinolide increased nitrate reductase activity and grain yield in the field in India. Annu. Appl. Biol. 143:121-4.

Jones RL (1972). Fractionation of the enzymes of the barley aleurone layer, evidence for a soluble mode of enzyme release. Planta. 103:95-109.

Klinich JF, Mandava NB, Todhunter JA, (1985).Relationship of nucleicacid metabolism to brassinolide induced responses in beans. J Plant Physiol. 120:207-214.

Maclachlan S, Zalik S (1963). Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley. Can. J. Bot. 41(7):1053-1062.

Pakistan agriculture research council. National Coordinated Pulses. Programme Narc, Islamabad.

Rao SSR, Vardhini BV, Sujatha E, Anuradha S (2002). Brassinosteroids-A new class of phytohormones. Current science. 82:1239-1245.

Ramraj VM, Vyas BN, Godrej NB, Mistry KB, Swami BN, Singh N, (1997). Effects of 28-homobrassinolide on yields of wheat, rice, groundnut, mustard, potato and cotton. J. Agri. Sci. 128:405-413.

Sairam RK (1994). Effects of Homobrassinolide application on metabolic activity and grain yield of wheat under normal and water-stress condition. J. of Agronomy and crop science. 173(1):11-16.

Sasse JM (2003). Physiological actions of brassinosteroids: An update. J. Plant Growth Reg. 22:276-288.

Sharma N, Hundal GS, Sharma I, Bhardwaj R (2014). Homobrassinolide alters protein content and activities of glutathione-S-transferase and polyphenol oxidase in *Raphanus sativus* L. plants under heavy metal stress. Toxicol. Int. 21:44-50.

Shamsul H, Ahmad A (2003). Brassinosteroids: Bioactivity and Crop Productivity. Kluwer Academic Publishers, Dordrecht.

Vardhini BV, Rao SSR (1998). Effect of brassinosteroids on growth, metabolite content and yield of arachis hypogaea. Phytochemistry. 48(6):927-930(4).

Zheng X, Van Huystee RB, (1992). Peroxidase regulated elongation of segments from peanut hypocotyls. Plant Sci. 81:47-56.

International Journal of Plant Physiology and Biochemistry

Related Journals Published by Academic Journals

- *African Journal Of Plant Science*
- *Journal Of Microbiology And Antimicrobials*
- *International Journal Of Biotechnology And Molecular Biology Research*
- *International Journal Of Biodiversity And Conservation*

academicJournals