



Digital Repository Universitas Jember

Journal of Basic and Applied  
Scientific Research  
(JBASR)

ISSN: 2090-4304

# Journal of Basic and Applied Scientific Research



Text Road Journals Publications

Volume  
Number  
January 2011

## Journal of Basic and Applied Scientific Research (JBASR) Monthly Publication



Number of issues per year: 12  
ISSN: 2090-4304 (Print)  
ISSN: 2090-424x (Online)

**Journal of Basic and Applied Scientific Research (JBASR)** is a peer reviewed, open access international scientific journal dedicated for rapid publication of high quality original research articles as well as review articles in the all areas of basic and applied sciences.

### Scope

**Journal of Basic and Applied Scientific Research (JBASR)** is devoted to the rapid publication of original and significant research in...

Accounts	Energy	Nuclear Engineering
Agricultural Sciences	Engineering, All Fields	Oceanography
Applied Biology	Entomology	Oncology
Biochemistry	Environment	Parasitology
Biological Sciences	Evolution	Petroleum & Gas
Biophysics	Fisheries	Pharmacology
Business and Economics	Food & Food Technology	Physics
Cell Biology	Genetics	Physiology
Chemical Engineering	Genomics	Plant Biology
Chemical Engineering	Geology	Population Biology
Chemistry	Immunology	Religious Studies
Civil Engineering	Infectious Diseases	Robotics

# Digital Repository Universitas Jember

Civil Engineering	Law	Signal Transduction
Commerce	Marine Sciences	Social Sciences
Communication & IT	Marine Technology	Solid State Technology
Computer Science	Mathematics Statistics	& Space Science
Construction	Medical Technology	Textile Industry & Fabrics
Dentistry	Medicine	Toxicology
Developmental Biology	Microbiology	Transportation
Ecology	Nanotechnology	Veterinary Science
Endocrinology	Neuroscience	Zoology



## **Journal of Basic and Applied Scientific Research**

Monthly Publication

### **INSTRUCTIONS TO AUTHORS**

#### **Submission**

Submit manuscripts as e-mail attachment to the Editorial Office at:

[info@textroad.com](mailto:info@textroad.com) or [textroadjournals@gmail.com](mailto:textroadjournals@gmail.com) along with covering letter. A manuscript number will be mailed to the corresponding author same day or within 48 hours. The authors may also suggest two to four reviewers for the manuscript (JBASR may designate other reviewers). There is no page limit. The submitting author takes responsibility for the paper during submission and peer review.

#### **Terms of Submission**

Papers must be submitted on the understanding that they have not been published elsewhere (except in the form of an abstract or as part of a published lecture, review, or thesis) and are not currently under consideration by another journal. The submitting author is responsible for ensuring that the article's publication has been approved by all the other coauthors. All enquiries concerning the publication of accepted papers should be addressed to [editor@textroad.com](mailto:editor@textroad.com).

**Journal of Basic and Applied Scientific Research will only accept manuscripts submitted as e-mail attachments.**

#### **Review Process**

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors within one week. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the JBASR to publish manuscripts within 4 weeks after submission.

#### **Style of Manuscripts**

Manuscripts should be written in clear, concise and grammatically correct English (with 10 font size and Times New Roman font style) so that they are intelligible to the professional reader who is not a specialist in any particular field. Manuscripts that do not conform to these requirements and the following manuscript format may be returned to the author prior to review for correction. The entire manuscript, including references, should be typed single spaced on one side of the paper. All pages should be numbered consecutively in the bottom centre starting from the title page. The manuscript should be presented in the following order.

#### **Title and Authorship Information**

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

#### **Abstract**

All manuscripts should not exceed 250-300 words and should describe the scope, hypothesis or rationale for the work and the main findings. Complete sentences, active verbs, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

#### **Keywords**

Key words (5-7 words) should be provided below the Abstract to assist with indexing of the article. These should not duplicate key words from the title.

#### **Introduction**

This section should include sufficient background information, provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. The aims of the manuscript should be clearly stated. The introduction should not contain either findings or conclusions. It should be understandable to colleagues from a broad range of scientific disciplines.

#### **Materials and Methods**

This should be complete enough to provide sufficient detail to allow the work to be repeated by others. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

#### **Results**

Results should be presented in a logical sequence in the text, tables and figures; repetitive presentation of the same data in different forms should be avoided. The results should not contain material appropriate to the Discussion. It should be written in the past tense when describing findings in the authors' experiments. Results should be explained, but largely without referring to the literature.

#### **Discussion**

The discussion should consider the results in relation to any hypotheses advanced in the Introduction and place the study in the context of other work. Results and Discussion sections can be combined.

## Conclusions

If an optional conclusion section is used, its content should not substantially duplicate the abstract.

## Acknowledgment

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

## References

Bibliographic references in the text appear like [1, 2, 5, 6], using square brace in superscript. References should be numbered consecutively, with style:

### Journal paper:

1. Hadjibabaie, M., N. Rastkari, A.Rezaie and M. Abdollahi, 2005. The Adverse Drug Reaction in the Gastrointestinal Tract: An Overview. *Intl. J. Pharmacol.*, 1 (1): 1-8.

### Books:

1. Daniel A. Potter, 2002. *Destructive turfgrass insects: Biology, diagnosis and control*. Wiley Canada Publishers, pp: 24-67.

### Chapters in Book:

1. Bray R.A., 1994. The leucaena psyllid. In: *Forage Tree Legumes in Tropical Agriculture* (eds R.C. Gutteridge and H.M. Shelton) pp. 283-291. CAB International, Oxford.

Titles of journals should be given in full. 'In press' can only be used to cite manuscripts actually accepted for publication in a journal. Citations such as 'manuscript in preparation' or 'manuscript submitted' are not permitted. Data from such manuscripts can only be mentioned in the text as 'unpublished data'.

### A Report:

1. Makarewicz, J.C., T. Lewis and P. Bertram, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. U.S. EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.

### Conference Proceedings:

1. Stock, A., 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.

### A Thesis:

1. Strunk, J.L., 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, M. S. thesis, Michigan State Univ., East Lansing, MI.

## Tables and Equations

Tables and equations should not be submitted in a format exceeding the A4 page size (in portrait form). **All tables should be embedded within the manuscript, and must be captioned and numbered sequentially.** Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text.

## Figures / Illustrations / Photographs

Graphics should be supplied as high resolution (at least 300-600 dp.i.) electronic files. Digital images supplied only as low-resolution print-outs cannot be used. Graphs, diagrams, chromatograms, photos, etc. should be prepared as clear, original positives, suitable for reproduction. **All figures should be embedded within the manuscript, and must be captioned and numbered sequentially.**

## Proofs

Proofs will be sent via e-mail as an Acrobat PDF file (e-mail attachment) and should be returned within 3 days of receipt. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

## Copyright and Permissions

By submitting a manuscript to the editor or publisher you are deemed to have granted permission to publish the manuscript and distribute it electronically or in any other form to different databases and abstracting services including libraries, universities and anywhere else. [**Copyright form**]

## Fees and Charges

Authors are required to pay a \$100 handling fee for publication of an article in the Journal of Basic and Applied Scientific Research. The Normal manuscript length is 10 pages, for every additional page 5 USD/page.

## Editorial Board

### Editor - in - Chief

#### **William Ebomoyi**

Ph.D., Professor, Department of Health Studies, College of Health Sciences, Chicago State University, **USA**.

E-mail: [editor@textroad.com](mailto:editor@textroad.com)

### Section Editors

#### **Prof. Dr. Sarwoko Mangkoedihardjo**

Professor, Professional Engineer of Indonesian Society of Sanitary and Environmental Engineers, **Indonesia**

#### **Saeid Chekani Azar**

PhD of Veterinary Physiology; Faculty of Veterinary, Department of Physiology, Ataturk University, Erzurum 25010, **Turkey**.

#### **Prof. Dr. Ashraf Latif Tadross**

Head of Astronomy Department, Professor of Star Clusters and Galactic Structure, National Research Institute of Astronomy & Geophysics (NRIAG), 11421 Helwan, Cairo, **Egypt**.

#### **Prof. Dr. Mounir M. Salem-Bekhet**

Associate Professor of Microbiology, Department of Pharmaceutics, King Saud University, **KSA**.

#### **Prof. Dr. Mario Bernardo-Filho**

Full Professor, Universidade do Estado do Rio de Janeiro, Head, Laboratório de Radiofarmácia Experimental, **Brazil**.

#### **Dr. Sandra Pacios Pujado**

University of Pennsylvania, Philadelphia, PA, **USA**.

#### **Vishal Patil, PhD**

Materials Research Laboratory, University of California, Santa Barbara, CA, **USA**.

#### **Dr. YUBAO CUI**

Associate Professor, Department of Laboratory Medicine, Yancheng Health Vocational & Technical College, Jiangsu Province, P. R. **China**

#### **Raja S Payyavula**

Research Associate, Bio Science Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, **USA**.

#### **Dr. Zhihong Song**

The Ames Laboratory of US DOE, 2238 MBB Iowa State University, IA 54411 **USA**.

#### **Prof. Dr. Nasser Fegh-hi Farahmand**

Associate professor, Department of Industrial Management, Tabriz Branch, Islamic Azad University, Tabriz, **Iran**

#### **Prof. Dr. Valdenir José Belinelo**

# Digital Repository Universitas Jember

Department of Health Sciences and Postgraduate Program in Tropical Agriculture, Federal University of Espirito Santo (UFES, São Mateus, ES, **Brazil**)

**Dr. Chandrasekar Raman**

Research Associate, Department of Biochemistry & Molecular Biophysics, Biotechnology Core Facility, 238, Burt Hall, Kansas State University, Manhattan 66506, KS, **USA**.

**Mr. Jiban Shrestha**

Scientist (Plant Breeding and Genetics), Nepal Agricultural Research Council, National Maize Research Program, Rampur, Chitwan, **Nepal**

**Dr. Nadeem Javaid**

Ph.D. (University of Paris-Est, France), Assistant Professor, Center for Advanced Studies in Telecommunications (CAST), COMSATS Institute of IT, Islamabad, **Pakistan**

**Dr. Syamkumar Siv Pillai**

Program Manager-National Clean Plant Network – Fruit Trees, Washington State University, **USA**

**Dr. Hala Ahmed Hafez Kandil**

Professor Assistant, National Research Centre, Plant Nutrition Department. Dokki, Giza, Cairo, **Egypt**

**Prof. Dr. Aziza Sharaby**

Pests and Plant Protection Department, National Research Center, Cairo, **Egypt**

**Prof. Dr. Sanaa T. El-Sayed**

Ex Head of Biochemistry Department, Professor of Biochemistry, Genetic Engineering & Biotechnology Division, National Research Centre, **Egypt**

**Dr. Pratap V. Naikwade**

M.Sc., Ph.D. Head, Department. of Botany, ASP College, Devrukh. Maharashtra, **India**.

**Shadia M. Abdel-Aziz**

Microbial Chemistry, National Research Center, **Egypt**

**Dr. Tarig Osman Khider**

Associate Professor, University of Bahri-Sudan, College of Applied and Industrial Sciences, Department of Pulp and Paper Technology, **Sudan**

**Dr. Hayman Z. Metwally**

Associate Professor of Space Science cairo University **Egypt** and Vice Dean of Quality Assurance and Development Hayel University **KSA**.

**Dr. Nawfal Jebbor**

Department of Physics, Moulay Ismail University, Meknes, **Morocco**.

**Dr. Eng. Ahmed Kadhim Hussein**

Assistant Professor, Department of Mechanical Engineering, College of Engineering, University of Babylon, **Republic of Iraq**.

**Prof. Dr. Abd El Fady Beshara Morcos**

Ass. Prof. of Relativistic Astrophysics and Cosmology, National Research Institute of Astronomy and Geophysics, **Egypt**.

# Digital Repository Universitas Jember

**Zohre Bahrami**

Shahid Beheshti University of Medical Sciences, Tehran, **Iran**. Researcher and Methodology Adviser.

**Dr. Ayhan Kapusuzoglu**

Department of Banking and Finance, Yildirim Beyazit University, **Turkey**.

**Dr. Charalambos Tsekeris**

Department of Psychology, Panteion University of Social and Political Sciences, Athens, **Greece**.

**Dr. Mahdi Zowghi**

Industrial and System Engineering, Management and Soft Computing, London Business and engineering School, **United Kingdom**.

**Dr. Tomislav Jurendic**

Bioquanta Ltd. for Research and Development, Koprivnica, **Croatia**

**Dr. Hanna Bolibok-Bragoszewska**

Warsaw University of Life Sciences, **Poland**.

**Prof. Md. Amin Uddin Mridha**

Ph.D. DIC (London), Plant Production Department, King Saud University, P.O.Box 2460, Riyadh 11451, **Kingdom of Saudi Arabia**

**Dr. Alaa Abdelwahed Abdelbary**

Prof. of Computational and Applied Mathematics, Arab Academy for Science and Technology & Maritime Transport, **Egypt**.

**Dr. N R Birasal**

Associate Professor, Zoology Department, KLE Society's G H College, HAVERI – 581 110, Karnataka state, **India**.

**Dr. Nawab Ali Khan**

Professor of Human Resource Management, College of Business Administration, Salman Bin Abdulaziz University, Post Box:165, Al Kharj - 11942 **Kingdom of Saudi Arabia**

**Prof. Dr. Amer A. Taqa**

DB'S. Department, College of Dentistry, Mosul University, **Iraq**.

**Deputy Section Editors****Prof. Dr. Tarek Ahmed Shokeir**

Professor and Consultant, Department of Obstetrics & Gynaecology, Fertility Care Unit, Mansoura University Teaching Hospitals, Mansoura Faculty of Medicine, **Egypt**

**Leila Falahati**

Department of Resource Management and Consumer Studies, Faculty of Human Ecology, University Putra **Malaysia**.

**Noorbakhsh Hooti**

Associate Professor in Dramatic Literature, Razi University, Faculty of Arts, English Department, Kermanshah, **Iran**

**Dr. Ali Elnaeim Musa**



# Digital Repository Universitas Jember

University of Bahri, Sudan College of Applied and Industrial Sciences, **Sudan**

**Prof. Dr. Magda M.A. Sabbour**

Professor , Department of Pests and Plant Protection- National Research Centre, Cairo, **Egypt.**

**Dr. Vahid Majazi Dalfard**

Department of Industrial Engineering, Islamic Azad University, Qazvin, **Iran**

**Dr. Mahboub Sheikhalizadeh Heris**

Assistant Professor, Department of Physical Education and Sport Sciences, Ahar Branch, Islamic Azad University, Ahar, **Iran**

**Dr. Basharia Abd Rub Alrasoul Abd Allah Yousef**

Deputy Dean at Faculty of Engineering, University of Bahri, Khartoum, **Sudan**

**Nasser Mousavi**

Islamic Azad University, Bilesavar Branch, **Iran.**

**Dr. Jinu John**

Associate Professor (Biotechnology), Jinu Bhavan, Chepra (P. O), Kottarakara, Kollam (Dist.), Kerala – 691520; **India.**

**Seyyed Mousa Hosseini**

Head of Young Researchers Club, Islamic Azad University (Sama Organization), Mazandaran and, Gilan province, **Iran.**

**Dr. Sunil Kumar**

Assistant Professor, Department of Mathematics, National Institute of Technology, Jamshedpur, 831014, Jharkhand, **India**

**Zairi Ismael Rizman**

Senior Lecturer, Faculty of Electrical Engineering, Universiti Teknologi MARA (UiTM) (Terengganu) **Malaysia**

**Muhammad Attique Khan Shahid,**

Associate Professor of Physics, Department of Physics, GC University, Faisalabad. **Pakistan.** PNRA certified Health Physicist, RPO, RSO Atomic and Nuclear Physics Lab

**Mohsen Shafiei Nikabadi(PhD.)**

Assistant Professor, Faculty of Economics and Management, Industrial Management Department, Semnan University, Semnan, **Iran.**

**Dr.Vuda Sreenivasarao**

Department of Computer and Information Technology, Defence University College, Deberzeit, **Ethiopia**

**Dr. Mohdammed Israil**

Post Doctoral Fellow, University Sains Malaysia, Pulau Penang, **Malaysia.**

**Dr. S. Ravichandran**

Assistant Professor, Department of Physics, Sathyabama University, **India**

**Dr. Hedayat Hosseinzadeh**

Department of Economics, Islamic Azad University, Tabriz, **Iran.**

**Dr. Sukumar Senthil Kumar**

School of Mathematical Sciences, Universiti Sains Malaysia, **Malaysia.**

# Digital Repository Universitas Jember

**Dr. Seyed Hossein Hosseini Nazhad**

Department of Computer Engineering, Islamic Azad University, Iran

**Seifedine Kadry**

American University of the Middle East, **Kuwait**.

**Dr. Datta Asaram Dhale**

Assistant Professor, Post Graduate Department of Botany, Ghogrey Science College, Dhule - Maharashtra State, **India**.

**Dr. Ho Soon Min**

Senior Lecturer, Faculty of Applied Sciences, INTI International University, Persiaran Perdana BBN, Putra Nilai, Negeri Sembilan, **Malaysia**.

**Dr. Ezzat Molouk Kenawy**

Economic Department, Faculty of Commerce, Kafr El-Sheikh University, **Egypt**.

**Dr. Farooq Ahmad Gujar**

Centre for Advanced Studies in Pure and Applied Mathematics, Bahauddin Zakariya University, Multan, 60800, **Pakistan**. & Head of Institution / Principal / Associate Professor of Mathematics.

**Alireza Karbalaee**

Assistant Professor, Department of English, Qeshm International Branch & Shariaty College, **Iran**.

**Dr. Seshadri Sekhar. Tirumala**

Principal, Chirala Engineering College, **India**.

**Sayed Roholla Mousavi,**

Department of Agriculture, Payame Noor University, Tehran, **Iran**.

**Dr. Tarek Y. El-Hariri**

Associated Professor, Egyptian Petroleum Research Institute, Exploration Department, **Egypt**.

**Dr Mamode Khan Naushad**

Department of Economics and Statistics, Faculty of social studies and humanities, University of Mauritius, **Mauritius**.

**Dhahri Amel**

Research professor, Research Unit: Materials, Energy and Renewable Energies (MEER)-Science Faculty of Gafsa, **Tunisia**.

**Dr. Muhammad Waqas Anwar**

COMSATS Institute of Information Technology, University Road, 22060, Abbottabad, **Pakistan**.

**Prof. Dr. Abdul-Kareem J. Al-Bermany**

Advance Polymer Laboratory, Physics Department/College of Science/Babylon University, **Iraq**.

**Dr. Bensafi Abd-El-Hamid**

Assistant Professor, Dept. of Chemistry, Faculty of Sciences, Abou Bekr Belkaid University of Tlemcen, **Algeria**.

**Dr. Vikas Anand Saharan**

Assistant Professor & Head, Department of Pharmaceutics, Institute of Pharmaceutical Sciences & Drug Research, Seth GL Bihani SD College of Technical Education, **India**.

# Digital Repository Universitas Jember

**Dr. Syed Zulfiqar Ali Shah**

Chairman Higher Studies and Research, Faculty of Management Sciences, International Islamic University Islamabad, **Pakistan.**

**Dr. Mohammad Reza Irvani**

Assistant Professor, Department: Social work, Azad University of Khomeinishahr, Islamic Azad University, Khomeinishahr branch, Khomeinishahr, Esfahan, **Iran.**

**Saima Anis Mustafa**

Assistant Professor in COMSATS Institute of Information Technology, University Road, Abbottabad, **Pakistan**

**Dr. Nagasamy Venkatesh**

Assistant Professor, Dept. of Pharmaceutics, JSS College of Pharmacy, Tamil Nadu, **India.**

**Mirza Hasanuzzaman**

Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, **Bangladesh.**

**Dr.K.V.L.N.ACHARYULU**

Faculty of Science, Department of Mathematics, Bapatla Engineering college, Bapatla, **India.**

**Maryam Ahmadian**

Post Doctoral Fellow, Department of Social and Development Sciences, Faculty of Human Ecology, Universiti Putra , UPM Serdang, Selangor, **Malaysia.**

**Dr. Mohammad Abul Hossain**

Associate Professor, Department of Chemistry, University of Dhaka, **Bangladesh.**

**Abdel Baset Hasoneh,**

PhD, Associate professor of Marketing, Head of marketing Department Al Isra University - Amman, **Jordan**

**Zahra Gharib Tarzeh**

Young Rsearch Club, Department of Business Management, Torbat-e-Jam Branch, Islamic Azad University, Torbat-e-Jam, **Iran**

**Dr. Muhammad Akram**

Faculty of Agriculture, Department of Eastern Medicine and Surgery, University of Poonch, Rawalakot, Azad Jamu and Kashmir, **Pakistan.**

**Dr. Anshoo Agarwal**

RAK Medical College and Health Sciences University, P.O.Box:13268, RAK, UAE, **United Arab Emirates**

**Dr. Aamir Shhazad**

Assistant Professor, Department of Physics, GC University, **Faisalabad**

**Dr.(Mrs.) Sunanda Sharma**

B.V.Sc & A.H., M.V.Sc., Ph.D. Department of Veterinary Gynecology and Obstetrics, College of Veterinary & Animal Science, Rajasthan University of Veterinary & Animal Sciences, Bikaner, **India.**

**Muhamad Fazil bin Ahmad**

Asst. Prof. Universiti Sultan Zainal Abidin, Terengganu, **Malaysia.**

# Digital Repository Universitas Jember

**Mohammad Hassan Boostani**

Education Organization of Fars Province & Young Researchers and Elites Club, Zarghan Branch, Islamic Azad University, Zarghan, **Iran**

**Naveed Ahmed**

Assistant Professor, Department of business administration, Indus International Institute, 2-Km, Jampur Road, Dera Ghazi Khan, **Pakistan**

**Ahmad Samariha**

PhD of Wood and Paper Engineering, Young Researches and Elites Club, Science and Research Branch, Islamic Azad University, Tehran, **Iran**.

**Rab Nawaz Lodhi**

PhD (ABD), Management Sciences (Bahria University Islamabad), Lecturer: Department of Management Sciences, COMSATS Institute of Information Technology, Sahiwal, **Pakistan**.

International Licensed Trainer - NVivo Qualitative Research: QSR International Limited **Australia**

**Dr. Majid Sharifi Rad**

Department of Range and Watershed Management, Faculty of Natural Resources, University of Zabol

**Dr. Muhammad Naeem**

LECTURER, Department of Information Technology, Hazara University, **Mansehra**.

**Dr. Sohrab Mirsaeidi**

Centre of Electrical Energy Systems (CEES), Faculty of Electrical Engineering (FKE), Universiti Teknologi Malaysia (UTM), 81310 Skudai, Johor, **Malaysia**

**Farhan Altaee**

Ministry of Science and Technology, **Iraq-Baghdad**

**Dr. Hafiz Abdul Wahab**

Assistant Professor of Mathematics, Department of Mathematics, Hazara University Mansehra **Pakistan**.

**Dr. Rohit Bansal**

Assistant Professor, Department of Management Studies, Vaish College of Engineering, Rohtak (Haryana), **India**

**Dr. Muhammad Akram**

Faculty of Agriculture, Department of Eastern Medicine and Surgery, University of Poonch, Rawalakot, Azad Jammu and Kashmir, **Pakistan**.

**Dr. Meena M.K.**

M.Sc.(Agri.), Ph.D., Assistant Professor, Department of Crop Physiology, University of Agricultural Sciences, Raichur-584104, Karnataka, **India**.

## List of Contents

Razali Musa, Azarudin Awang

### **Historical Review of Issues on Takfir in Malaysia**

*J. Basic Appl. Sci. Res.* 2015 5(2): 1-5. [[Abstract](#)] [[Full Text PDF](#)]

---

Mohammad Mohamadi, Amir Hossein Abadi, Ebrahim Taghizadeh, Jafar Amanolahi, Shirin Ahmadi Dastjerdi

### **A Survey on the Claim's Conditions of Direct Action (DA)**

*J. Basic Appl. Sci. Res.* 2015 5(2): 6-13. [[Abstract](#)] [[Full Text PDF](#)]

---

Mohib Ur Rahman, Irfan Ullah and Khalil Jebran

### **Effects of Government Expenditure on Private Investment: Evidence from Pakistan**

*J. Basic Appl. Sci. Res.* 2015 5(2): 14-23. [[Abstract](#)] [[Full Text PDF](#)]

---

Popy Hartatie Hardjo, Nurul Holifah, Tri Handoyo, Win Darmanto and Bambang Sugiharto

### **Production of Polyclonal Antibodies against Sucrose Transporter (SUT1) Protein Expressed in Escherichia coli BL21 and Application for Immunodiagnosis**

*J. Basic Appl. Sci. Res.* 2015 5(2): 24-30. [[Abstract](#)] [[Full Text PDF](#)]

---

P.Ajitha, E.Chandra

### **A Survey on Outliers Detection in Distributed Data Mining for Big Data**

*J. Basic Appl. Sci. Res.* 2015 5(2): 31-38. [[Abstract](#)] [[Full Text PDF](#)]

---

Muhammad Ali, Shen Lei, Atiq Ur Rehman, Amna Anjum

### **Relationship of Strategic Human Resource Management Practices with Organization Performance and Employee Relation Climate**

*J. Basic Appl. Sci. Res.* 2015 5(2): 39-50. [[Abstract](#)] [[Full Text PDF](#)]

---

## Production of Polyclonal Antibodies against Sucrose Transporter (SUT1) Protein Expressed in *Escherichia coli* BL21 and Application for Immunodiagnosis

Popy Hartatie Hardjo<sup>1)</sup>, Nurul Holifah<sup>2)</sup>, Tri Handoyo<sup>3)</sup>, Win Darmanto<sup>4)</sup>  
and Bambang Sugiharto<sup>2)</sup>

<sup>1)</sup>The Faculty of Biotechnology, University of Surabaya, Surabaya

<sup>2)</sup>Biology Department, Faculty of Mathematics and Science, University of Jember, Jember

<sup>3)</sup>Faculty of Agrotechnology, University of Jember, Jember

<sup>4)</sup>Biology Department, Faculty of Science and Technology, Airlangga University, Surabaya

Received: September 16, 2014

Accepted: January 10, 2015

### ABSTRACT

Sucrose transporter (SUT1) protein plays important roles in sucrose translocation from leaves to other organs in plants, therefore it is interested to study the existence of SUT1 protein in plants. Detection of SUT1 protein in plants can be done by using specific antibodies for that protein. This research was done to prepare SUT1 polyclonal antibodies by using SUT1 recombinant protein produced in *Escherichia coli* strain BL21. The production of SUT1 recombinant protein was done using fragment cDNA-*SoSUT1* of sugarcane plants inserted inside plasmid pET28a, and expressed as a fusion protein containing N-terminal hexa-histidine tags. Expressed SUT1 was purified under denaturing conditions by affinity chromatography. Recombinant protein was purely used as antigen and was injected subcutanly in the back of female New Zealand White rabbits. *Western Blot* analysis using SUT1 polyclonal antibodies could detect the existence of SUT1 recombinant protein until the concentration 1.0 ng. Those antibodies could detect the existence of SUT1 protein at sugarcane plants.

**KEYWORDS:** polyclonal antibodies, SUT1 recombinant protein, cDNA – *SoSUT1*.

### INTRODUCTION

Sucrose is one of the most common and abundant carbon forms in plants. Most plants synthesize sucrose as a major photosynthetic product and use it for long distance carbon transport. Therefore sucrose transport in plants probably is highly regulated and sucrose transporters have indispensable roles in the regulation.

The sucrose translocation process from photosynthesis tissue to storage tissue in the plants was done with the help of sucrose transporter protein (SUT) as the intermediary. This protein was known as the indicator of the amount of sucrose which could be accumulated in plants [1]. Considering the importance of protein role in sucrose translocation, some researchers successfully isolated cDNA-*SUT* from different kinds of plants, such as potatoes and tomatoes [2], tobacco [1], Arabidopsis [3], rice [4] and sugarcane plants [5,6].

To study sucrose translocation process on plants, SUT1 protein analysis needed to be done. Plants protein detection could be done with several methods, such as double diffusion [7], Western blot and immunohistochemistry [8] using specific antibodies. Direct isolation and purification of SUT1 protein from plants was difficult because the amount of SUT1 protein in plants was very low and it was located in cell membrane.

cDNA-*SoSUT1* in sugarcane plants [6] could be used to produce SUT1 recombinant protein through transformation and the expression in *E.coli*. Moreover, SUT1 recombinant protein would be used to produce SUT1 polyclonal antibodies in rabbits.

Recently, pET28a vector could be used for protein expression in *E.coli* efficiently. In this plasmid, there were hexa-histidine tags at the edge of N-terminal to make the protein purification easily. On the other hand, this plasmid was occupied with efficient promoter, so DNA inserted can be transcribed and translated easily. By inserting cDNA-*SoSUT1* in pET28a, SUT1 protein was expected to be expressed, so it could be isolated for the purpose of antibodies production.

The availability of SUT1 antibodies would be used to determine the amount of SUT1 protein using Western blot method, so sucrose translocation process in plants could be studied. The knowledge of this sucrose translocation process was the important discovery to increase the translocation and the amount of sucrose in plants.

### MATERIALS AND METHODS

#### Expression of recombinant protein

The construction of pET28a-*SoSUT1* was transformed into *E. coli* BL21 and transformants were screened by antibiotic medium, restriction enzyme analysis and PCR using *SoSUT1* primers forward 5'CATATGGTACCATACAGAGGA'3 and reverse 5'GTAAGTTGCTTCCAGAGCTC3' The PCR reaction

consisted of one cycle at 94 °C for 2 min, 30 cycles at 94 °C for 30 s, 55 °C for 60 s and 72 °C for 2 min, and a final extension step at 72 °C for 7 min. The amplified product was analyzed by 1% agarose gel electrophoresis. Recombinant plasmids were extracted from two clones using the Roche *High Pure Plasmid Isolation* kit and sequenced using the same primers for colony screening in order to confirm the integrity of the ORF. A single clone, containing pET28a-*SoSUT1* was selected for expression studies. A single clone of *E.coli* BL21 contained with the construction of pET28a-*SoSUT1* plasmid [9] were grown in 4 mL of liquid LB medium contained with 35 mg/L chloramphenicol and 50 mg/L kanamycin in shaker incubator (37°C 150 rpm) overnight and it was used as starter. 4 mL starter culture was inoculated to 200 mL of liquid LB medium contained with 35 mg/L chloramphenicol and 50 mg/L kanamycin; and it was incubated for 3 hours until it reached optical density (OD<sub>600nm</sub>) 0.7, then 0.5 mM inducer isopropyl-β-D-thiogalactopyranoside (IPTG) was added and it was incubated for 5 hours at 37 °C on shaker, 150 rpm.

#### Verification and localization of expressed recombinant protein

Cells were harvested by using 5000 rpm microcentrifugation for 10 minutes at 4 °C. The pellets were resuspended in NPI-10 buffer pH 8 (50mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl and 10 mM imidazole) and 100 µg/mL lysozyme was added. The pellets was sonicated for 3 minutes and centrifuged (12000 rpm) at 4 °C for 20 minutes. Supernatant (soluble fraction) was taken to examine the existence of SUT1 recombinant protein. Pellet, which contained cell debris (insoluble fraction), was resuspended with NPI -10 buffer and centrifuged at 12000 rpm, at 4°C for 20 minutes. Supernatant was thrown away and pellet resuspended with DNPI-10 buffer pH 8.0 (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl and 10 mM imidazole and 8 M urea) and sonicated for 3 minutes to diffuse the membrane protein. Suspension was centrifuged at 12000 rpm, 20 °C for 20 minutes and supernatant obtained (insoluble fraction) were analyzed. Soluble and insoluble fraction were analyzed for SDS-PAGE to find the location of the expression of SUT1 recombinant protein.

#### Purification of recombinant SUT1 protein

The SDS – PAGE analysis was done with the concentration of 15% akrilamid for *separating gel* which contained 30% akrilamid, Tris – HCl pH 8.8, SDS 10%, 50 µL ammonium persulfate (APS) and 5 µL N, N, N', N'-tetramethylethylenediamine (TEMED) and the concentration of 4.5 % akrilamid for stacking gel 30% akrilamid, Tris – HCL pH 6.8, SDS 10% and 4.5 µL TEMED [10]. After the location of SUT1 recombinant protein expression being discovered at insoluble fraction, supernatant purification (insoluble fraction) was done with the column of Ni – NTA resin affinity chromatography. The resin which was ready to use was placed in a chromatography column and equilibrated with DNPI – 10 buffer. Purification result of SUT1 recombinant protein was analyzed by SDS – PAGE to check the protein purity, and if it was still found contaminant protein, the cutting of the gel and the protein electroelusion would be done. Urea was removed by dialyzing buffer of *phosphate buffer saline* (PBS) pH 7.4 contained with 8 g NaCl, 0.2 g KCl, 1.44 g Na<sub>2</sub>HPO<sub>4</sub>, 0.24 g KH<sub>2</sub>PO<sub>4</sub> in 1 L buffer for 12 hours with constant stirring at 4 °C. The following steps, after 12 hours, the buffer was replaced with a new same buffer. The concentration of refolded protein was measured by the Lowry method [11] using bovine serum albumin (BSA) as a standard. The protein was aliquoted before lyophilization [12] and stored at -20 °C.

#### Production and evaluation of polyclonal antibody raised against recombinant SUT1 protein

The production of SUT1 polyclonal antibodies was done by injecting SUT1 recombinant protein (antigene) in female New Zealand white rabbits. A week before injection, pre-immune blood serum was taken from veins of the rabbits' ears. Injection was done by mixing SUT1 recombinant protein (0.5 mg) with *Freund's Complete Adjuvant* (FCA) (1 : 1) until it was homogeneous, and then it would injected subcutaneously at the rabbits' back. After 2 weeks, *booster* injected was implemented by mixing the antigen of SUT1 recombinant protein (0.1 mg) with *Freund's Incomplete Adjuvant* (FIA) (1 : 1) until it was homogeneous. It was done continuously once a week until 9 weeks [13].

#### Ouchterlony analysis

Ouchterlony analysis was done by dissolving agarose 1 % with *agarose solution buffer* which contained 0.5 M Tris – HCl, 0.1 M EDTA, NaCl and 0.1 M NaN<sub>3</sub>. The solution was heated in *microwave oven* until it was homogeneous, then it was poured in *glass plate* evenly. The solution was left to be cold and frozen, then the well was made with diameter of 2 – 3 mm and the distance between each well was 0.5 cm. Antigen and antibodies solution were put inside well side by side and they were incubated for 2 days and were observed. The precipitin line formed between well of antibodies and antigen were stained with *Coomassie Brilliant Blue* (CBB) 0.1 %.

#### Western blot analysis

The samples of SUT1 recombinant protein with the concentration of 1 ng, 10 ng, 100 ng and 1000 ng were analyzed using SDS – PAGE. The separated proteins were electroblotted onto nitrocellulose membrane using Semi-dry Trans-Blot at 180 mA for 2.5 hours. The membrane was washed 3 times using Tris Buffer Saline (TBS, 25 mM Tris-Cl pH 7.5, 150 mM NaCl, 3 mM KCl) for 5 minutes each. After being washed, protein in the membrane was blocked by submerging it in 2 % non-fat powdered milk in TBS for 30 minutes. Then, membrane was submerged in TBS contained with 2 % non-fat powdered milk and primary antibodies was given (SUT1 polyclonal antibodies) with the dilution of 2000x, incubated overnight with the gentle shaking. Membrane was rewashed 3 times with TBS for 5 minutes each, then it was given secondary antibodies, goat anti-rabbit IgG alkaline phosphatase (AP)-conjugate in TBS non-fat powdered milk 2 % and incubated for an hour at room temperature. The bands of interest were visualized by reaction with freshly prepared substrate: 5-bromo-4-chloro 3-indolyl-phosphate (BCIP, 168\_g/mL) and nitroblue tetrazolium (NBT, 332\_g/mL), in developing buffer (0.1M Tris buffer, pH 9.5, containing 0.1M NaCl and 5 mM MgCl<sub>2</sub>). The protein which could be detected in certain concentration showed the sensitivity of antibodies titer.

### The detection of SUT1 protein in plants

Crude leaf extracts were prepared by grinding the leaf tissue in 3 mL extraction buffer (50 mM MOPS - NaOH pH 7.5, 10 mM MgCl<sub>2</sub>.6H<sub>2</sub>O, 1 mM EDTA, 2.5 mM dithiothreitol (DTT), 10 μM phenyl methyl sulfonyl fluoride (PMSF), and 10% polyvinylpyrrolidone (PVP). Crude leaf extracts was centrifugated 12000 rpm at 4°C for 10 minutes. The pellet was resuspended with the same buffer for 3 times, then the pellets were resuspended with 150 μL extraction buffer (50 mM Tris-base pH 8.5, 1 mM EDTA, 2% SDS, 30% sucrose, 5 mM DTT), and was centrifugated 12000 rpm for 10 minutes at 4°C. Supernatant was stored in -80 °C to be analyzed with SDS-PAGE and Western blot.

## RESULTS AND DISCUSSION

The existence of *pE28a-SoSUT1* in *E.coli* BL21 was confirmed by restriction enzyme analysis and PCR. *E.coli* grown in solid LB medium and contained 35 mg/L chloramphenicol antibiotic and 50 mg/L kanamisin proved that those *E.coli* contained *pET28a-SoSUT1* plasmid. Restriction enzyme analysis which used *Xba*I and *Xho*I was intentionally used to prove that *pET28a-SoSUT1* had contained cDNA-*SoSUT1* fragment (Fig.1) that 2 DNA band in the size of 5017 bp and 352 bp were obtained. PCR analysis used a couple of F/R *SUT1* primer to prove that cDNA-*SoSUT1* fragment was inserted at *pET28a-SoSUT1* as shown in Fig. 2. It was obtained DNA band with the size of 255 bp which was cDNA-*SoSUT1* fragment. One of *E.coli* clones which contained *pET28a-SoSUT1* construction, was then used for the production SUT1 recombinant protein. In Fig. 3, new protein with the size of 15 kDa was emerged at *E.Coli* insoluble fraction and it contained *pET281-SoSUT1*, however, that protein was not found in soluble fraction of *pET28a* and *pET28a-SoSUT1*, and also control insoluble fraction of *pET28a*.

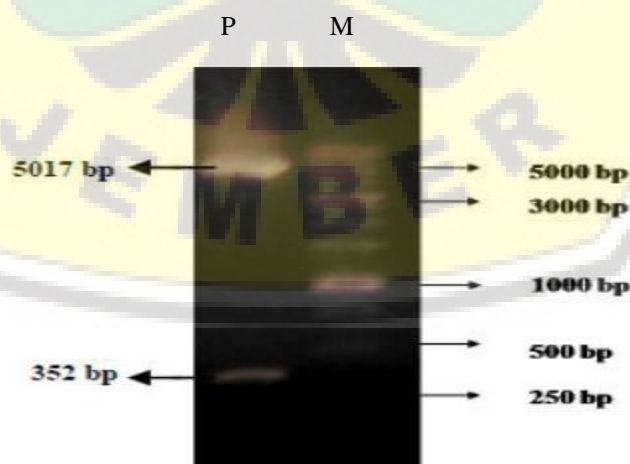


Fig. 1. Nucleotide fragment as the result of *pET28a-SoSUT1* restriction plasmid. *pET28a-SoSUT1* which had been cut using *Xho*I and *Xba*I produce 2 nucleotide fragments with the size of 5017 bp and 352 bp.

P: *pET28a-SoSUT1*, M: protein marker



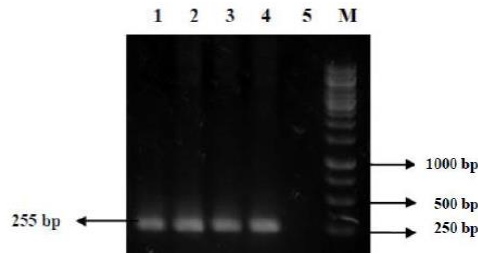


Fig. 2. Product of PCR *E.coli* which contained pET28a-SoSUT1 construction used a couple of SUT1 primer. Lane 1, 2, 3, 4: *E.coli* clones which contained pET28a-SoSUT1 construction. Lane 5: *E.coli* negative control contained pET28a. M: DNA marker.

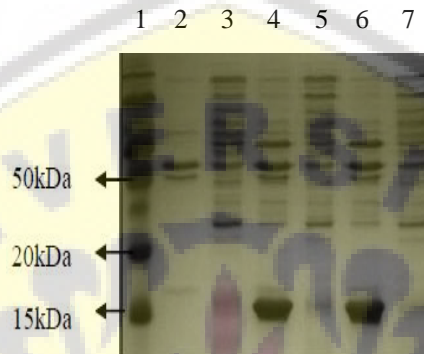


Fig. 3. SDS-PAGE analysis expressed fraction protein of soluble and insoluble at *E.coli*. Lane 1: protein marker, lane 2: protein of insoluble fraction pET28a *E.coli*, lane 3: protein of soluble fraction pET28a *E.coli*, lane 4 and 6: protein of insoluble fraction pET28a-SoSUT1 *E.coli*, lane 5 and 7: protein of soluble fraction pET28a-SoSUT1 *E.coli*.

The purification result showed that SUT1 recombinant protein, which had been fused with hexa-histidine tag had been successfully purified by using Ni-NTA resin. Basically, Ni-NTA resin which contained Nickel ion ( $\text{Ni}^{2+}$ ) would be connected to protein which had fusion protein hexa-histidine tag. That bond could be eluted by imidazole with the high concentration of 100 – 250 mM. This was intended to release protein which contained hexa-histidine tag which was attached to  $\text{Ni}^{2+}$  ion. The contamination of other protein (Fig. 4A) was probably caused by washing before elution, which was not done maximum enough, therefore there was still non target protein engaged in elution. Furthermore, protein electroelution was done towards the contaminated one in order to obtain the pure protein (Fig. 4B). Protein electroelution was an easy method to isolate protein from polyacrylamide gel using electricity [14]. The purification result using this method was specific in which there was only one protein band of SUT1 target with the size of 15 kDa. The purification result was specific in electroelution because only target protein band was cut and removed using electricity.

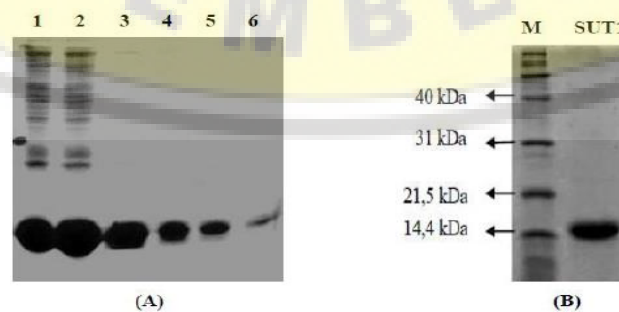


Fig. 4. SDS PAGE analysis of SUT1 recombinant protein which was washed (A) and SUT1 recombinant protein after electroelution (B). A(lane1-2) SUT1 protein which was still mixed with other protein at the beginning of the washing. A (lane 3-6) SUT1 protein washed in resin and separated from other protein. B SUT1: SUT1 recombinant protein after electroelution. M: protein marker.

Based on *ouchterlony* test (Fig. 5), serum taken for the first time (Ab1) at the 4<sup>th</sup> week after the first injection or the 1<sup>st</sup> week after *booster*, SUT1 antibodies was still not detected, it could be seen because there was not precipitin line. SUT1 antibodies inside rabbit had actually been formed because the first injection of antigen would stimulate B cells and formed antibodies and memory cells called primary response. Antibodies formed in primary response was still little and would increase in the next antigen injection, called secondary response [15]. SUT1 antibodies were detected in serum secondly taken at the 5<sup>th</sup> week after the first injection and at the 2<sup>nd</sup> week after *booster*. However, serum formed still had low titer, it could be seen from the thin line of precipitin. On the 6<sup>th</sup> week until the 7<sup>th</sup> week, antibodies titer increased, while on the 8<sup>th</sup> week until the 9<sup>th</sup> week, it was most likely to decrease. Precipitin line formed in *Ouchterlony* analysis showed there was match in the bond of antigen and antibodies and it was migrated one another inside gel. This precipitin reaction was specific because only the match antibodies and antigen which could be bond. This method was often used to check the specificity of polyclonal antibodies [16].

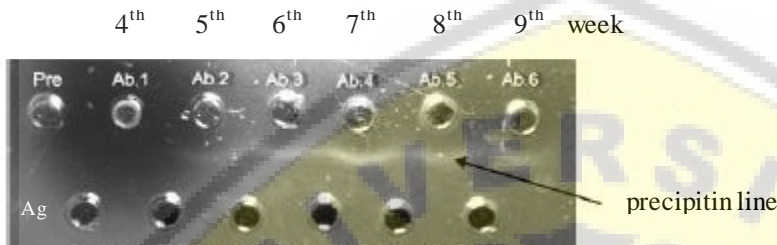


Fig. 5. Ouchterlony analysis of SUT1 polyclonal antibodies and SUT1 recombinant protein antigen. Pre : pre-immunization serum; Ab1 – Ab 6 : weekly serum (the 4<sup>th</sup> week until the 9<sup>th</sup> week after the first injection). Ab: antibody, Ag: antigen (SUT1 recombinant protein)

Based on Western blot analysis (Fig. 6), SUT1 polyclonal antibodies of Ab4 serum could detect the existence of SUT1 antigen until the concentration 1 ng with antibodies dilution 1 : 2000. As seen in the result, it could be concluded that SUT1 polyclonal antibodies had high titer because it could detect the existence of antigen with the very low concentration, such as 1 ng.

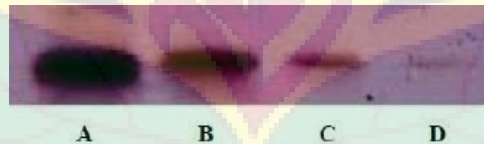


Fig. 6. Sensitivity test of the capability of SUT1 polyclonal antibodies using Western blot analysis with several concentrations of SUT1 recombinant protein antigen. A: 1000 ng, B: 100 ng, C: 10 ng, D: 1 ng

SUT 1 protein detection on plants was done with Ouchterlony and Western blot analysis using sugar- cane protein as antigen. The result of Ouchterlony analysis showed the precipitine line formed between antibodies and insoluble fraction. SUT1 polyclonal antibodies could bind SUT1 protein at insoluble fraction (Fig.7) and it indicated that the protein existed on cell membrane . In soluble fraction, antibodies – antigen bond reaction was not happened, so precipitin line was not formed. This was suitable with the report of [17] that sucrose transporter which was isolated from sugar beet leaves existed on cell membrane.

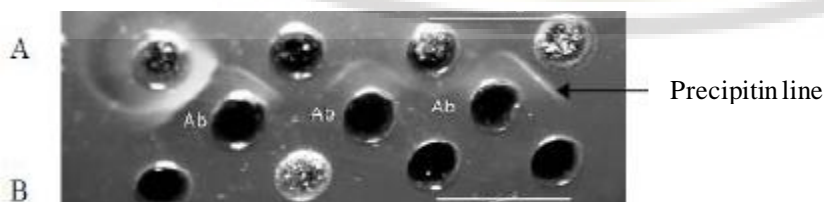


Fig. 7. Ouchterlony analysis of SUT1 protein on sugarcane plants using serum of the 4<sup>th</sup> SUT1 polyclonal antibodies. A: insoluble fraction. B: soluble fraction ; precipitin line formed at insoluble fraction. Ab: antibody

The SUT1 polyclonal antibodies has been used successfully for the detection of SUT1 protein on sugarcane leaves by Western blot analysis (Fig. 8), and specific SUT1 protein band is detected with the size of 65 kDa.

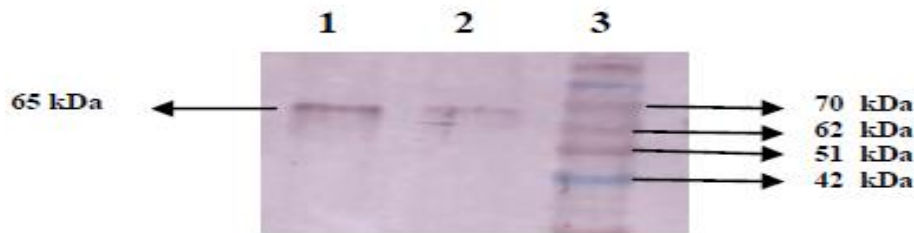


Fig. 8. Western blot analysis of SUT1 protein on sugarcane leaves with SUT1 polyclonal antibodies. Lane 1 and 2: SUT1 protein, lane 3: protein marker.

### CONCLUSION

SUT1 recombinant protein, which was the result of cDNA-*SoSUT1* fragment overexpression on *E.coli* BL 21 cells, could be used to produce SUT1 polyclonal antibodies in rabbits. The result of polyclonal antibodies against SUT1 recombinant protein can be applied successfully for SUT1 efficient detection in plants.

### ACKNOWLEDGEMENT

This work was supported by Grant Fundamental Research, Dikti, Indonesia. 2011 (on the behalf of Prof. Bambang Sugiharto, Ph.D.).

### REFERENCES

1. Lemoine, R., B. Lukas, B. Laurence, S. Soulaïman, K. Christina, R. Matthieu, G. Cecile, D. Serge, and B.F. Wol, 1999. Identification of a pollen-specific transporter-like protein *NtSUT3* from tobacco. *FEBS Letters*, 454:325-330.
2. Riesmeier, J.W., B. Hirner, W.B. Frommer, 1993. Potato sucrose transporter expression in minor veins indicates a role in phloem loading. *The Plant Cell*, 5 :1591-1598.
3. Weise, A., Barker, L., Kuhn, C., Lalonde, S., Buschmann, H., Frommer, W.B., and J.M. Ward, 2000. A new subfamily of sucrose transporters, SUT4, with long affinity/high capacity localized in enucleate sieve elements of plants. *Plant Cell*, 12:1345 – 1355.
4. Matsukura, C.A., T. Saitoh, T. Hirose, R. Obsugi, P. Perata, J. Yamaguchi, 2000. Sugar uptake and transport in rice embryo: expression of companion cell-specific sucrose transporter (*OsSUT1*) induced by sugar and light. *Plant Physiology*, 124: 85-93.
5. Rae, AL., JM. Perroux, CPL. Grof, 2005. Sucrose partitioning between vascular bundles and storage parenchyma in the sugarcane stem. A potential role for the *ShSUT1* sucrose transporter. *Planta*, 220:817-825.
6. Sugiharto, B., 2009. Isolasi *full length SoSUT1*, Laporan Penelitian Hibah Kompetensi (no published).
7. Cheung, H., K. Chan, and C. Cheng, 2002. Production of polyclonal antibody against recombinant goldfish prolactin and demonstration of its usefulness in a non-competitive antigen-capture ELISA. *Comp. Biochem. Physiol. Biochem. Mol. Biol.*, 131: 37-46.
8. Hackell, N., N. Schauer, F. Carrari, A.R. Fernie, B. Grimm, and C. Kuhn, 2006. Sucrose transporter *LeSUT1* and *LeSUT2* inhibition affects tomato fruit development in different ways. *Plant Journal*, 45: 180–192.
9. Sugiharto, B., M. Hazmi, Slameto, dan P. Dewanti, 2010. Peningkatan produksi gula melalui overekspresi gen *sucrose phosphate synthase* dan *sucrose transporter* pada tanaman tebu (*Saccharum* spp. hybrids). Laporan Penelitian Hibah Kompetensi (no published).
10. Garfin, D.E., 1990. One-dimensial Gel Electrophoresis. In M.P. Deutscher (Ed.) *Guide to Protein Purification*. *Methods in Enzymology*, 182: 425-441.

11. Pohl, T., 1990. Concentration of Protein and Removal of Solutes. In M.P. Deutscher (Ed.) Guide to Protein Purification. Methods in Enzymology, 182: 68-83.
12. Dunbar, BS. and E.D. Schwoebel, 1990. Preparation of Polyclonal Antibodies. In M.P. Deutscher (Ed.) Guide to Protein Purification. Methods in Enzymology, 182:663-669.
13. Harrington, M.G., 1990. Elution of protein from gel. In M.P. Deutscher (Ed.) Guide to Protein Purification. Methods in Enzymology, 182: 448-495.
14. Abbas, A. K., A.H. Lichtman, and S. Pillai, 1994. Cellular and molecular immunology; Updated Edition. Elsevier Inc. p. 107-158.
15. Kunkel, J.G., 1988. Immunological techniques in insect biology. Gilbert & Miller Eds. p. 1-49.
16. Lemoine, R., 2000. Sucrose transporter in plants: update on function and structure. *Biochimica et Biophysica Acta Biomembranes*, 1465: 246-262.

