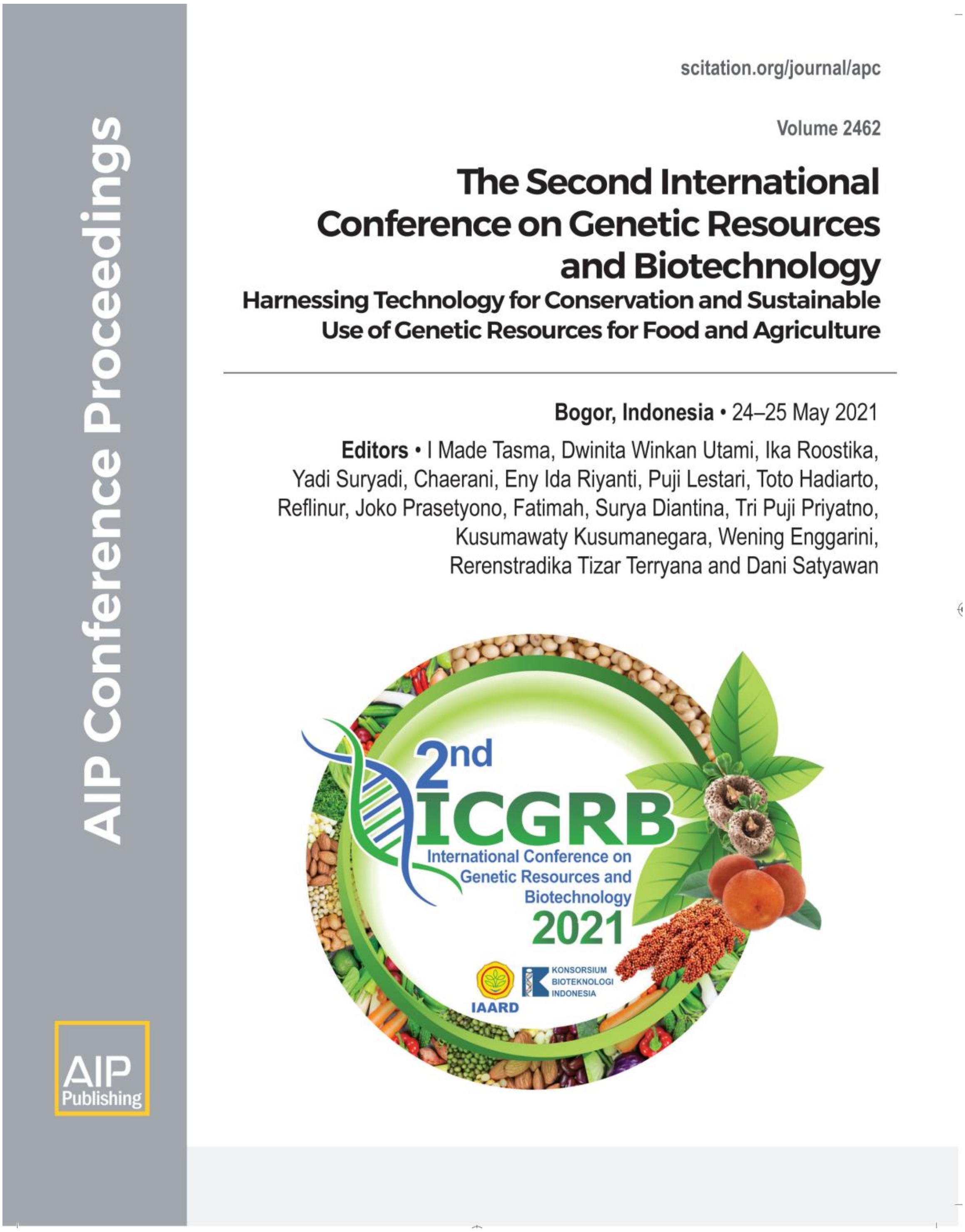

January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

Cite as: AIP Conference Proceedings **2462**, 010002 (2022); https://doi.org/10.1063/12.0008934
Published Online: 19 January 2022

Steering, Scientific and Organizing Committees

Steering Committee

Chair:

Dr. Ir. Fadjry Djufry, M.Si , *Director General of Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia*

Vice Chair:

Ir. Mastur Ph.D, *Director of Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Ministry of Agriculture, Indonesia*

Dr. Siswa Setyahadi, *Head of Indonesian Biotechnology Consortium, Indonesia*

Secretary:

Dr. Sustiprijatno, *Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Ministry of Agriculture, Indonesia*

Scientific Committee

Prof. Dr. M. Sabran, *Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Ministry of Agriculture, Indonesia*

Prof. Dr. Bahagiawati A., *Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Ministry of Agriculture, Indonesia*

Prof. Endang Gati Lestari, *Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Ministry of Agriculture, Indonesia*

Associate Prof. Endang Septiningsih, *Texas A & M University, USA*

Dr. Prakit Somta, *Kasetsart University, Thailand*

Dr. Laosatit Kularb, *Kasetsart University, Thailand*

Dr. Back Ki Kim, *Seoul National University, South Korea*

Dr. Iswari Saraswati Dewi, *Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Ministry of Agriculture, Indonesia*

Dr. Puji Lestari, *Indonesian Center for Rice Research, IAARD, Ministry of Agriculture, Indonesia*

Dr. Dani Satyawan, *Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Ministry of Agriculture, Indonesia*

Dr. Alina Akhdiya, *Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Ministry of Agriculture, Indonesia*

Ir. Tri Puji Priyatno, PhD, *Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Ministry of Agriculture, Indonesia*

Dr. Ir. Ragapadmi Purnamaningsih, *Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Ministry of Agriculture, Indonesia*

Dr. Nurul Hidayatun, *Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Ministry of Agriculture, Indonesia*

The Second International Conference on Genetic Resources and Biotechnology
AIP Conf. Proc. 2462, 010002-1–010002-3; https://doi.org/10.1063/12.0008934
Published by AIP Publishing. 978-0-7354-4172-9/$30.00

Organizing Committee

Chair	:	Dr. Toto Hadiarto
Vice Chair	:	Dr. Dani Satyawan
Secretary	:	Dr. Wening Enggarini
		Rerenstradika Tizar Terryana, M.Si
Treasurer	:	Dra. Sih Parmiyatni
		Ma'sumah, S.Si
Publication and documentation:		
		Dr. Hakim Kurniawan
		Endo Kristiyono, M.T.I.
		Andika Bakti, S.I.Kom
		Ansori
Logistic	:	Wawan, M.Si.
		M. Hasni Zulfikar

Preface: The Second International Conference on Genetic Resources and Biotechnology

Cite as: AIP Conference Proceedings **2462**, 010001 (2022); https://doi.org/10.1063/12.0006897
Published Online: 19 January 2022

Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24^{th}-25^{th} of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

The Second International Conference on Genetic Resources and Biotechnology
AIP Conf. Proc. 2462, 010001-1–010001-1; https://doi.org/10.1063/12.0006897
Published by AIP Publishing. 978-0-7354-4172-9/$30.00

Table of Contents

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

PREV

Conference date: 24–25 May 2021
Location: Bogor, Indonesia
ISBN: 978-0-7354-4172-9
Editors:
I Made Tasma, Dwinita Winkan Utami, Ika Roostika, Yadi Suryadi, Chaerani, Eny Ida Riyanti, Puji Lestari, Toto Hadiarto, Reflinur, Joko Prasetyono, Fatimah, Surya Diantina, Tru Puji Priyanto, Kusumawaty Kusumanegara, Wening Enggarini, Rerenstradika Tizar Terryana and Dani Satyawan
Volume number: 2462
Published: Jan 19, 2022
DISPLAY :

- 20
- 50
- 100
- all

PRELIMINARY

AIP Conference Proceedings **2462**, 010001 (2022); https://doi.org/10.1063/12.0006897

-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Committees: The Second International Conference on Genetic Resources and Biotechnology

AIP Conference Proceedings **2462**, 010002 (2022); https://doi.org/10.1063/12.0008934

-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

EFFECTIVE MANAGEMENT OF CONSERVATION AND SUSTAINABLE USE OF GENETIC RESOURCES FOR FOOD AND AGRICULTURE

FreeJanuary 2022

Harnessing plant genetic resources through biotechnology for food security in Indonesia

Mastur, Reflinur, Nurul Hidayatun, Sustiprijatno, Fatimah, Tri Puji Priyatno and Puji Lestari
AIP Conference Proceedings **2462**, 020001 (2022); https://doi.org/10.1063/5.0075671

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

DNA barcoding of *Vatica bantamensis*, a critically endangered tree endemic to Banten, Indonesia

Muhammad Rifqi Hariri, Iyan Robiansyah, Dipta Sumeru Rinandio, Dodo, Desi Siti Sundari, Cecep H. Sukmawan and Bayuntoro Ardi
AIP Conference Proceedings **2462**, 020002 (2022); https://doi.org/10.1063/5.0075529

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Genetic parameters of agronomic traits in soybean (*Glycine max* [L.] Merrill) genotypes tolerant to drought

Made J. Mejaya, Suhartina, Purwantoro, Novita Nugrahaeni and Titik Sundari
AIP Conference Proceedings **2462**, 020003 (2022); https://doi.org/10.1063/5.0075159

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Yield stability performance of soybean (*Glycine max* [L.] Merrill) lines tolerant to drought

Suhartina, Purwantoro, Novita Nugrahaeni, Abdullah Taufiq and Made Jana Mejaya
AIP Conference Proceedings **2462**, 020004 (2022); https://doi.org/10.1063/5.0075158

- SHOW ABSTRACT
-
  - PDF
  - E-READER

- ADD TO FAVORITES
- SHARE
- EXPORT CITATION

FreeJanuary 2022

Polymorphisms and associations of the *RACK1* genes with antibody response to Newcastle disease in KUB chickens

Ifa Manzila, Puji Lestari, Tike Sartika, Tri Puji Priyatno, Risa Indriani, Kristianto Nugroho and Rerenstradika Tizar Terryana
AIP Conference Proceedings **2462**, 020005 (2022); https://doi.org/10.1063/5.0075622

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Rice grain quality evaluation of promising lines of rice under irrigation and for salinity tolerance

Dody D. Handoko, Nafisah, Aris Hairmansis, Trias Sitaresmi, Heni Safitri, Satoto, Ali Imamuddin, Cucu Gunarsih and Untung Susanto
AIP Conference Proceedings **2462**, 020006 (2022); https://doi.org/10.1063/5.0075956

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Existing diversity profile for kernel characteristics of maize germplasm in IAARD-ICABIOGRAD gene bank

Andari Risliawati, Sobir, Trikoesoemaningtyas, Willy B. Suwarno and Puji Lestari
AIP Conference Proceedings **2462**, 020007 (2022); https://doi.org/10.1063/5.0075178

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Characterization of Japansche citroen rootstock somaclones and *in vitro* selection for aluminium tolerance

Deden Sukmadjaja, Mia Kosmiatin and Tiwi Wati
AIP Conference Proceedings **2462**, 020008 (2022); https://doi.org/10.1063/5.0077888

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Resistance to brown planthoppers (*Nilaparvata lugens* Stål) in rice accessions originated from Sumatra Island, Indonesia

Dodin Koswanudin, Nurul Hidayatun and Muhamad Ace Suhendar
AIP Conference Proceedings **2462**, 020009 (2022); https://doi.org/10.1063/5.0075680

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Morphological identification of underutilized local fruits in Kutai Barat Regency to support their conservation and sustainable use

Fitri Handayani, Nurbani and Asep Pebriandi
AIP Conference Proceedings **2462**, 020010 (2022); https://doi.org/10.1063/5.0075594

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Genetic resources of adlay (*Coix lacryma-jobi* L.) in East Kalimantan as source of functional food

Fitri Handayani, Muhammad Amin and Muhammad Taufiq Ratule
AIP Conference Proceedings **2462**, 020011 (2022); https://doi.org/10.1063/5.0075593

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Screening of soybean genotypes resistance to rust disease (*Phakopsora pachyrhizi*)

Sumartini and Kurnia Paramita Sari
AIP Conference Proceedings **2462**, 020012 (2022); https://doi.org/10.1063/5.0075674

- SHOW ABSTRACT

-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Identification of soybean promising lines resistant to pod-sucking bug, *Riptortus linearis* (Fabricius)

M. Muchlish Adie, Titik Sundari, Kurnia Paramita Sari and Ayda Krisnawati
AIP Conference Proceedings **2462**, 020013 (2022); https://doi.org/10.1063/5.0075343

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Variation in pod shattering resistance among black soybean genotypes associated with agronomic traits

Ayda Krisnawati, Titik Sundari and M. Muchlish Adie
AIP Conference Proceedings **2462**, 020014 (2022); https://doi.org/10.1063/5.0075338

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Preliminary characterization and identification of genetic integrity of velvet bean germplasm in IAARD-ICABIOGRAD gene bank

Nurwita Dewi, Andari Risliawati and Nurul Hidayatun
AIP Conference Proceedings **2462**, 020015 (2022); https://doi.org/10.1063/5.0076355

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Plant parasitic nematodes infesting three minor legumes (velvet bean, lablab bean, and jack bean)

Chaerani, Try Zulchi P. Hariyadi and Nurwita Dewi
AIP Conference Proceedings **2462**, 020016 (2022); https://doi.org/10.1063/5.0075204

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Proactive management approach of seed PGRFA conservation during the pandemic of coronavirus disease (COVID-19) in Indonesia

Nurul Hidayatun, Andari Risliawati, Nurwita Dewi, Lina Herlina and Dodin Koswanudin
AIP Conference Proceedings **2462**, 020017 (2022); https://doi.org/10.1063/5.0075531

- SHOW ABSTRACT

-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Evaluation of mung bean accessions in saline soil based on quantitative morphological characters

Trustinah, Ratri Tri Hapsari, Rudi Iswanto and Rudy Soehendi
AIP Conference Proceedings **2462**, 020018 (2022); https://doi.org/10.1063/5.0075324

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Screening and evaluation of 100 upland rice accessions for developing high-yielding upland rice varieties tolerant against acid soil

Lina Herlina and Yusi N. Andarini
AIP Conference Proceedings **2462**, 020019 (2022); https://doi.org/10.1063/5.0075550

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Morphological characters of sugarcane mutant (*Saccharum officinarum* L.) from *in vitro* selection for drought stress

Rr. Sri Hartati, Sri Suhesti and Nurya Yuniyati
AIP Conference Proceedings **2462**, 020020 (2022); https://doi.org/10.1063/5.0075656

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Identifying potential seedless citrus accessions through floral structure and pollen performance

Baiq Dina Mariana, Anis Andrini and Sri Andayani
AIP Conference Proceedings **2462**, 020021 (2022); https://doi.org/10.1063/5.0076922

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Secondary characters based selection of Indonesian kenaf (*Hibiscus cannabinus* L.) germplasm for developing superior varieties

Taufiq Hidayat R. S., Marjani, Nurindah, Muhammad Rasyidur Ridho, Cynthia Lestari Hertianti and Widya Fatriasari
AIP Conference Proceedings **2462**, 020022 (2022); https://doi.org/10.1063/5.0075716

- SHOW ABSTRACT

-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Genetic relationship of pigmented rice (*Oryza sativa* L.) collected from Eastern Indonesia based on morpho-agronomical traits and SSR markers

Yusi Nurmalita Andarini, Willy Bayuardi Suwarno, Hajrial Aswidinnoor and Hakim Kurniawan
AIP Conference Proceedings **2462**, 020023 (2022); https://doi.org/10.1063/5.0075706

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Rejuvenation and morphological characterization of local rice from the province of Yogyakarta

Setyorini Widyayanti, Sutarno, Endang Wisnu Wiranti and Kristamtini
AIP Conference Proceedings **2462**, 020024 (2022); https://doi.org/10.1063/5.0075721

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Characterization of plant architecture and yield trait of castor (*Ricinus communis* L.) germplasm suitable for mechanical harvesting

Tantri Dyah Ayu Anggraeni and Rully Dyah Purwati
AIP Conference Proceedings **2462**, 020025 (2022); https://doi.org/10.1063/5.0075155

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Characterization and interrelationships of the number of vessel bundles with yield components in various genotypes of soybean (*Glycine max* [L.] Merrill)

Anna S. Karyawati and Dyah P. Fitrawantio
AIP Conference Proceedings **2462**, 020026 (2022); https://doi.org/10.1063/5.0075693

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Tuber starch content of edible canna (*Canna indica* L.) from different geographical origins

Surya Diantina, Randy Sanjaya, Kristina Dwi Atmini, Ace Suhendar and Dodin Koswanudin
AIP Conference Proceedings **2462**, 020027 (2022); https://doi.org/10.1063/5.0075922

- SHOW ABSTRACT

-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

The diversity of morpho-agronomic characters and identification of early maturity cassava (*Manihot esculenta* Crantz.) germplasm

Tinuk Sri Wahyuni, Kartika Noerwijati and Made J. Mejaya
AIP Conference Proceedings **2462**, 020028 (2022); https://doi.org/10.1063/5.0075658

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Radiosensitivity and phenotypic characterization of gamma ray-induced mutant population of four *Capsicum annuum* L. cultivars grown in screen house

Andri Fadillah Martin, Dyah Retno Wulandari, Tri Muji Ermayanti, Betalini Widhi Hapsari, Erwin Al Hafiizh and Laela Sari
AIP Conference Proceedings **2462**, 020029 (2022); https://doi.org/10.1063/5.0075173

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Morphological performances of mutant butterfly pea (*Clitoria ternatea* L.)

Try Zulchi, Ali Husni, Dwinita Wikan Utami, Reflinur, Mia Kosmiatin, Tarkus Suganda and Agung Karuniawan
AIP Conference Proceedings **2462**, 020030 (2022); https://doi.org/10.1063/5.0075592

- SHOW ABSTRACT
-
    - PDF
    - E-READER
    - ADD TO FAVORITES
    - SHARE
    - EXPORT CITATION

FreeJanuary 2022

Screening of beta carotene and its correlation with tuber flesh color in sweet potato

Kristina Dwi Atmini, Surya Diantina, Muhamad Sabda and Dodin Koswanudin
AIP Conference Proceedings **2462**, 020031 (2022); https://doi.org/10.1063/5.0075618

- SHOW ABSTRACT
-
    - PDF
    - E-READER
    - ADD TO FAVORITES
    - SHARE
    - EXPORT CITATION

FreeJanuary 2022

Evaluation of morpho-agronomical characters and grain quality of red rice lines

Heni Safitri and Puji Lestari
AIP Conference Proceedings **2462**, 020032 (2022); https://doi.org/10.1063/5.0078807

- SHOW ABSTRACT
-
    - PDF
    - E-READER

- ADD TO FAVORITES
- SHARE
- EXPORT CITATION

FreeJanuary 2022

Growth variation and relationship of clove progenies of high-yielding mother trees collected from various regions in Indonesia

Mariana Susilowati, Sri Wahyuni, Adi Setiadi and Nurliani Bermawie
AIP Conference Proceedings **2462**, 020033 (2022); https://doi.org/10.1063/5.0075824

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Screening on bast fiber plants resistant to spiral stem borer, *Agrilus acutus* (Coleoptera: Buprestidae)

Sujak, Nurindah, Dwi Adi Sunarto, Marjani and Nurul Hidayah
AIP Conference Proceedings **2462**, 020034 (2022); https://doi.org/10.1063/5.0075691

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Characteristic of indigenous *Leuconostoc mesenteroides* EN 17-11 protease and its stability during storage at cold and freezing temperatures

Tatik Khusniati, Ika, Harry Noviardi and Sulistiani
AIP Conference Proceedings **2462**, 020035 (2022); https://doi.org/10.1063/5.0076004

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Performance of introduced lines based on morphological markers for diversity enrichment of Indonesian chili pepper (*Capsicum annuum* L.) varieties

Rinda Kirana, Catur Hermanto, Reflinur and Derek W. Barchenger
AIP Conference Proceedings **2462**, 020036 (2022); https://doi.org/10.1063/5.0075186

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

The growth of garlic internal sprout on different storage condition

Chotimatul Azmi, Imas Rita Saadah, Nazly Aswani and Asih Kartasih Karjadi
AIP Conference Proceedings **2462**, 020037 (2022); https://doi.org/10.1063/5.0075180

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Genetic diversity analysis of *Castanopsis argentea* using random amplified polymorphic DNA markers

Muhammad Imam Surya, Lily Ismaini, Decky Indrawan Junaedi, Aisyah Handayani, Taufikurrahman Nasution, Muhammad Efendi, Andes Hamuraby Rozak, Zaenal Mutaqien, Musyarofah Zuhri, Imawan Wahyu Hidayat, Fitri Kurniawati, Vandra Kurniawan, Dwinda Mariska Putri and Risha Amilia Pratiwi
AIP Conference Proceedings **2462**, 020038 (2022); https://doi.org/10.1063/5.0077390

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

APPLICATION OF GENOMICS AND MOLECULAR MARKERS FOR GENETIC RESOURCE CONSERVATION AND CROP ADAPTATION TO CLIMATE CHANGE

FreeJanuary 2022

Current status of tidal swamp rice varieties and its improvement for Fe toxicity tolerance and biofortification

Muhamad Sabran, Dwinita Wikan Utami and Susilawati
AIP Conference Proceedings **2462**, 030001 (2022); https://doi.org/10.1063/5.0075202

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Agroforensic, a new emerging study using molecular marker technique

Edy Listanto, Ahmad Warsun, Ahmad Dadang, Eny Ida Riyanti, Saptowo Jumali Pardal, Sustiprijatno and Mastur
AIP Conference Proceedings **2462**, 030002 (2022); https://doi.org/10.1063/5.0075164

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Molecular diversity comparison in local rice accessions originated from Kalimantan and other islands of Indonesia

Puji Lestari, Rerenstradika Tizar Terryana, Kristianto Nugroho, Andari Risliawati, Nurul Hidayatun, Priatna Sasmita, Yudhi Sastro, I. Gusti Komang Dana Arsana and Ikhwani
AIP Conference Proceedings **2462**, 030003 (2022); https://doi.org/10.1063/5.0075665

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Genetic variation of Adan, a Krayan local rice mutant, using microsatellite markers

Joko Prasetiyono, Tio Fadel Rafsanjani, Tri Aminingsih, Tasliah and Sugiono Moeljopawiro
AIP Conference Proceedings **2462**, 030004 (2022); https://doi.org/10.1063/5.0075660

- SHOW ABSTRACT

-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

The genome sequence of Ciherang, an Indonesian rice mega variety, revealed the footprints of modern rice breeding

Ida Rosdianti, Dani Satyawan, Muhamad Yunus and Dwinita Wikan Utami
AIP Conference Proceedings **2462**, 030005 (2022); https://doi.org/10.1063/5.0075676

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Field adaptation and molecular characterization of Code-*qTSN4* and Code-*qDTH8* rice lines at two different locations

Tasliah, Kurniawan Rudi Trijatmiko and Joko Prasetiyono
AIP Conference Proceedings **2462**, 030006 (2022); https://doi.org/10.1063/5.0075661

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Hybrid purity assessment in F_1 hybrids segregating for phytophthora root rot resistance genes of chili pepper (*Capsicum annuum* L.)

Fatimah, Reflinur, Joko Prasetiyono, Wartono, Kristianto Nugroho, Rinda Kirana, Dani Satyawan, Rerenstradika Tizar Terryana, Aqwin Polosoro, Puji Lestari and I. Made Tasma
AIP Conference Proceedings **2462**, 030007 (2022); https://doi.org/10.1063/5.0075160

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Characterization of genomic variation on three Indonesian oil palm genotypes analyzed using next-generation sequencing HiSeq

I. Made Tasma, Habib Rijzaani, Dani Satyawan, Ida Rosdianti, Edy Supriyanto and Razak Purba
AIP Conference Proceedings **2462**, 030008 (2022); https://doi.org/10.1063/5.0075392

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Cytological and molecular identifications of seedless tangerine derived from endosperm culture

Chaireni Martasari, Mia Kosmiatin, Ali Husni, Kurniawan Budiarto and Innez Candri Gilang Purnama
AIP Conference Proceedings **2462**, 030009 (2022); https://doi.org/10.1063/5.0076395

- SHOW ABSTRACT

Improvement of sex determination of salak plant using sequence characterized amplified regions

Reflinur, Ma’sumah, Namira Nur Arfa, Budi Setiadi Daryono and Azis Natawijaya
AIP Conference Proceedings **2462**, 030010 (2022); https://doi.org/10.1063/5.0075698

Table of Contents

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Conference date: 24–25 May 2021
Location: Bogor, Indonesia
ISBN: 978-0-7354-4172-9
Editors:

I Made Tasma, Dwinita Winkan Utami, Ika Roostika, Yadi Suryadi, Chaerani, Eny Ida Riyanti, Puji Lestari, Toto Hadiarto, Reflinur, Joko Prasetyono, Fatimah, Surya Diantina, Tru Puji Priyanto, Kusumawaty Kusumanegara, Wening Enggarini, Rerenstradika Tizar Terryana and Dani Satyawan
Volume number: 2462
Published: Jan 19, 2022
DISPLAY :

- 20
- 50
- 100
- all

APPLICATION OF INNOVATIVE CROP IMPROVEMENT TECHNIQUES FOR CONSERVATION AND SUSTAINABLE USE OF PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE

FreeJanuary 2022

Design and *in vitro* test of sgRNA for the CRISPR/Cas9 plasmid construct of the *SQS* gene of *Artemisia annua* L.

Sri Koerniati
AIP Conference Proceedings **2462**, 040001 (2022); https://doi.org/10.1063/5.0075695

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

The efficacy of genetically modified (GM) corn Bt11 against *Ostrinia furnacalis* (Guenee) and *Helicoverpa armigera* (Hubner)

Bahagiawati and Diani Damayanti
AIP Conference Proceedings **2462**, 040002 (2022); https://doi.org/10.1063/5.0075312

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Construction and introduction of OsAER1::LeAlaAT cassette to improve the nitrogen use efficiency in rice cv. Mekongga

Atmitri Sisharmini, Aniversari Apriana, Intan Kamila, Aqwin Polosoro, Wening Enggarini, Tri Joko Santoso, Toto Hadiarto, Bahagiawati A. Husin and Kurniawan Rudi Trijatmiko
AIP Conference Proceedings **2462**, 040003 (2022); https://doi.org/10.1063/5.0075458

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Environmental safety assessment of genetically engineered potato resistant to late blight caused by *Phytophthora infestans*

Alberta Dinar Ambarwati, Eny Ida Riyanti, Edy Listanto, Tri Joko Santoso, Toto Hadiarto and Kusmana
AIP Conference Proceedings **2462**, 040004 (2022); https://doi.org/10.1063/5.0075612

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES

- SHARE
- EXPORT CITATION

FreeJanuary 2022

Backcrossing of soybean lines containing aluminium tolerance gene into superior soybean variety, Biosoy

Saptowo J. Pardal, Amalia Prihaningsih, Suharsono, Ratna Utari and Riri Sundasari
AIP Conference Proceedings **2462**, 040005 (2022); https://doi.org/10.1063/5.0075187

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Phenotypic and genetic stability evaluation of the targeted *GA20ox-2* gene mutation in CRISPR/Cas9 mutant rice derived from Mentong cultivar

Aniversari Apriana, Tri Joko Santoso, Atmitri Sisharmini, Reflinur, A. Dinar Ambarwati, Toto Hadiarto, Sustiprijatno and Nuryati
AIP Conference Proceedings **2462**, 040006 (2022); https://doi.org/10.1063/5.0075603

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Transformation of *csp* gene into tobacco plant mediated by *Agrobacterium tumefaciens*

Sustiprijatno, Seagames Waluyo and Suharsono
AIP Conference Proceedings **2462**, 040007 (2022); https://doi.org/10.1063/5.0075571

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

PLANT CELL AND TISSUE CULTURE FOR CONSERVATION AND EFFECTIVE UTILIZATION OF GENETIC RESOURCES

FreeJanuary 2022

The application of gamma ray irradiation to increase triterpenoid compounds in embryogenic calli of *Centella asiatica* L. Urban

Ika Roostika, Suci Rahayu and Nurliani Bermawie
AIP Conference Proceedings **2462**, 050001 (2022); https://doi.org/10.1063/5.0076402

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

The effect of $FeSO_4$ concentration on the callus growth of two chili (*Capsicum annuum* L.) varieties

Rossa Yunita, Endang Gati Lestari, Iswari S. Dewi, Mastur and Bambang Sapta Purwoko
AIP Conference Proceedings **2462**, 050002 (2022); https://doi.org/10.1063/5.0075223

- SHOW ABSTRACT
-
  - PDF

- E-READER
- ADD TO FAVORITES
- SHARE
- EXPORT CITATION

FreeJanuary 2022

Evaluation of ratooning ability in several sweet sorghum (*Sorghum bicolor* [L.] Moench) mutant lines

Endang Gati Lestari, Iswari Saraswati Dewi, Rossa Yunita and Amin Nur
AIP Conference Proceedings **2462**, 050003 (2022); https://doi.org/10.1063/5.0075542

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Response of gamma ray irradiation derived-cultures of three sugarcane varieties to drought stress induced by polyethylene glycol

Ragapadmi Purnamaningsih and Suci Rahayu
AIP Conference Proceedings **2462**, 050004 (2022); https://doi.org/10.1063/5.0075185

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Sucrose and putrescine increased callus induction in tomato anther culture

Iswari Saraswati Dewi, Imam Nur Kholis, Bambang Sapta Purwoko and Ratna Ningsih
AIP Conference Proceedings **2462**, 050005 (2022); https://doi.org/10.1063/5.0075666

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Field evaluation of elephant grass mutant lines (*Pennisetum purpureum* Schumach.) in highlands

Ali Husni, Muhammad Rifay, Mia Kosmiatin and Vyta W. Hanifah
AIP Conference Proceedings **2462**, 050006 (2022); https://doi.org/10.1063/5.0076418

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Increasing drought tolerance of sugarcane through gamma ray irradiation and *in vitro* selection

Sri Suhesti, Syafaruddin, I. Ketut Ardana, Endang Hadipoentyanti and Rr. Sri Hartati
AIP Conference Proceedings **2462**, 050007 (2022); https://doi.org/10.1063/5.0076155

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Cells density affects cell production of *Citrus limonia* in flask and air-lift bioreactor cultures and limonin farming

Dita Agisimanto, Farida Yulianti and Hidayatul Arisah
AIP Conference Proceedings **2462**, 050008 (2022); https://doi.org/10.1063/5.0075651

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

THE USE OF MICROBIAL GENETIC RESOURCES AS BIOLOGICAL CONTROL AGENTS OF AGRICULTURAL PESTS AND DISEASES, AND FOR SOIL BIOREMEDIATION

FreeJanuary 2022

In Silico functional prediction of CAS2, a protein specifically expressed in appressorium and required for pathogenicity of *Colletotrichum gloeosporioides*

Tri Puji Priyatno, Farah Diba Abu Bakar, Rohaiza Ahmad Redzuan, Abdul Munir Abdul Murad and Ifa Manzila
AIP Conference Proceedings **2462**, 060001 (2022); https://doi.org/10.1063/5.0075625

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Biofertilizer increases nutrient use efficiency (NUE) of nitrogen, phosphorus, and potassium at leaves level of *Artemisia annua* L.

Wiguna Rahman, Arthur A. Lelono, Erwin Al Hafiizh and Tri Muji Ermayanti
AIP Conference Proceedings **2462**, 060002 (2022); https://doi.org/10.1063/5.0075503

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Effect of nitrogen fixation and phosphate solubilizing bacteria on growth and yield of lowland rice in different soil type

Ikhwani, Higa Afza, Siti Yuriyah and Waluyo
AIP Conference Proceedings **2462**, 060003 (2022); https://doi.org/10.1063/5.0077914

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Morphological, physiological, and molecular identification and characterization of yeast isolated from Indonesian fruits and woods

Rerenstradika Tizar Terryana, Nazhirotul Ilmiyah, Inda Setyawati, Titin Haryati, Karden Mulya, Eny Ida Riyanti, Yudi Sastro and Puji Lestari
AIP Conference Proceedings **2462**, 060004 (2022); https://doi.org/10.1063/5.0075170

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

The effect of coating application using chitosan enzymatic depolymerization on anthracnose disease suppression in mango (*Mangifera indica* L.) cv. ‘Arumanis’

Yadi Suryadi, Dwi Ningsih Susilowati, I. Made Samudra, Alina Akhdiya and Karsinah
AIP Conference Proceedings **2462**, 060005 (2022); https://doi.org/10.1063/5.0075183

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Understanding yeast tolerance as cell factory for bioethanol production from lignocellulosic biomass

Eny Ida Riyanti and Edy Listanto
AIP Conference Proceedings **2462**, 060006 (2022); https://doi.org/10.1063/5.0075157

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Isolation and pathogenicity test of fusarium basal rot and purple blotch fungal pathogens from shallot and *Allium* spp

Chaerani, Ragapadmi Purnamaningsih and Suci Rahayu
AIP Conference Proceedings **2462**, 060007 (2022); https://doi.org/10.1063/5.0075209

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Morphological characters and efficacy of thirteen entomopathogenic fungi of *Aschersonia aleyrodis* Webber isolates on whitefly (*Bemisia tabaci* Gennadius)

Yusmani Prayogo, Marida Santi Yudha Ika Bayu, Sri Wahyuni Indiati and Made Jana Mejaya
AIP Conference Proceedings **2462**, 060008 (2022); https://doi.org/10.1063/5.0076067

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Physicochemical characteristics of yoghurt from various beans and cereals

Heny Herawati, Diana Nur Afifah, Eni Kusumaningtyas, Sri Usmiati, Agus S. Soemantri, Miskiyah, Elmi Kamsiati and Muchamad Bachtiar
AIP Conference Proceedings **2462**, 060009 (2022); https://doi.org/10.1063/5.0075712

- SHOW ABSTRACT

-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

The potential use of zeolite and exopolysaccharide bacteria for reduction of degradation and carbon emission on oil palm plantation in tropical peatland

Laksmita P. Santi, Haryo T. Prakoso and Donny N. Kalbuadi
AIP Conference Proceedings **2462**, 060010 (2022); https://doi.org/10.1063/5.0075506

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

FreeJanuary 2022

Application of phosphate solubilizing microbes to promote the effectiveness of rock phosphate on cacao seedling growth in acid soil

Kurnia Dewi Sasmita, Iswandi Anas, Syaiful Anwar, Sudirman Yahya and Gunawan Djajakirana
AIP Conference Proceedings **2462**, 060011 (2022); https://doi.org/10.1063/5.0075843

- SHOW ABSTRACT
-
  - PDF
  - E-READER
  - ADD TO FAVORITES
  - SHARE
  - EXPORT CITATION

Cells density affects cell production of *Citrus limonia* in flask and air-lift bioreactor cultures and limonin farming

Cite as: AIP Conference Proceedings **2462**, 050008 (2022); https://doi.org/10.1063/5.0075651
Published Online: 19 January 2022

Dita Agisimanto, Farida Yulianti and Hidayatul Arisah

Cells Density Affects Cell Production of *Citrus limonia* in Flask and Air-Lift Bioreactor Cultures and Limonin Farming

Dita Agisimanto[a)], Farida Yulianti and Hidayatul Arisah

Indonesian Citrus and Subtropical Crops Research Institute, IAARD,
Jln. Raya Tlekung No.1, Junrejo, Batu 65327, East Java, Indonesia

[a)]Corresponding author: agisimanto@gmail.com

Abstract. Japansche citroen (JC) is popular citrus rootstock in Indonesia due to well-adapted to biotic and abiotic stresses. JC is naturally rich of secondary metabolites. Therefore, cells production of JC through tissue culture is potential for continuous metabolites farming in local pharmaceutical industries. The objectives of study were to to determine the best initial cells density (ICD) in flask and air-lift bioreactor (ALB) cultures for mass cells proliferation and to determine the limonin content in cells. Callus was induced from nucellus and habituated callus were collected to initiate cell suspensions. Selected cells were transferred to flask and ALB cultures at the density of 2, 4, and 6 mg/ml for 90 days of culturing. An increasing ICD affected cell growth in flask and ALB cultures. Cells in flask culture grew slower and were produced lower in quantity than in ALB. At 4 mg/ml, growth rate (GR) of cells in flask culture at day 30 reached maximum of 4.12 mg/day, 87-folds lower than in ALB that reached 360 mg/day. Limonin at 120.725 µg/g dried cells (DW) were harvested from ALB within 30 days, while total phenolic was 9.883±0.471 mg GAE/g DW and total flavonoid was 0.144±0.011 mg QE/g DW. This study confirmed the advantages of cell culture as a perpetual source for production of biomass content metabolites that amenable lower operational cost compared to natural production for local industries.

INTRODUCTION

Citrus is one of the most important fruit in the world for human health due to their active properties. Citrus fruit possess a range of phenolic compounds, alkaloids, flavonoids, terpenes, limonoids, phytonutrients and vitamins C, carotenoids, and essential oils [1]. Limonoid is one of the important compound in citrus. It is a family of polycyclic secondary metabolites [2] that are commonly found in seeds [3] and fruits of pummelos, grapefruits, lemons, sweet oranges, and mandarins [4], peels of ponderosa lemon *Citrus pyriformis* Hassk [5], *C. jambhiri* Lush [6], bergamot (*C. bergamia* Risso) [7], rind and pulp of *C. limon* fruit [8]. About 50 limonoids aglycones and glucosides and even more have been isolated from diverse citrus species, such as *C. aurantium*, *C. limon*, *C. reticulata*, *C. sudachi*, *C. unshiu*, *C. paradisi*, *C. sinensis*, *C. jambhiri*, and *C. pyriformis* [9], and *C. limonia* Osbeck (Japansche citroen/JC) [10]. *C. limonia* cv. Rangpur lime (JC) is popular rootstock [11]. In fact, broaden adaptation of rootstock is driven by its metabolites compound [11].

Limonoids has been found largely in Rutaceae and Meliaceae [9]. The name limonoid comes from limonin, the first bitter tetranortriterpenoid isolated from citrus [12]. Research interest on limonoid from citrus is due to its contribution to the bitter taste of citrus fruits, which harms the global citrus fruit and juice industry [13]. However, it's diverse bio-activities evaluated *in vitro* and *in vivo* possess several biological activities, including antitumor, neuroprotective, immunomodulatory, insecticidal, anti-obesity, anti-hyperglycemic activities [13], analgesic [14], anticancer such as liver cancer [15], breast cancer [16], intestinal carcinogenesis [17], colon cancer [18], anti-inflammatory [13], antibacterial [13], antioxidant [6], antiviral [13], antimalarial [9], and larvicidal [19], prevent non-alcoholic fatty liver, cardiovascular diseases, type-2 diabetes mellitus, and metabolic syndromes [20]. As food-

The Second International Conference on Genetic Resources and Biotechnology
AIP Conf. Proc. 2462, 050008-1–050008-8; https://doi.org/10.1063/5.0075651
Published by AIP Publishing. 978-0-7354-4172-9/$30.00

derived phytochemicals, limonoids from citrus confirmed to have well tolerated by non-cancerous cell lines [2], animal models [21], and human body [22]. Therefore, limonoids present potential applications in food and pharmaceutical industries, functional food additives, pesticides, and feed additives [13]. Recently, molecular docking studies of phytochemicals against the therapeutic protein targets of SARS-CoV-2 supported the effective binding affinity with limonin, a triterpenoid found in citrus [23]. The highest level of genomic similarity between SARS-CoV and SARS-CoV-2 [24], and the effectiveness of triterpenoids against SARS-CoV prompted us to search potential phytochemicals from limonoids and triterpenoids [25].

Production of agricultural based product that safe to environment, biodiversity, and quality of agricultural crops are referred to sustainable crop production [26]. Tissue culture of plant (PTC) offers remarkable prospects to produce desirable plant structures, such as cells [27], shoots [28], root [29], and a variety of secondary metabolites inside the cells in large quantities [30] for drugs, cosmetics, and food. Extracted material derived from plant cell and tissue cultures were extensively used as component of 50 products and brought a new wave in PTC technology [27].

The PTC have been utilized to produce seedling of *C. reticulata* Blanco cv. Madu [31], Oncidium 'Sugar Sweet' [32], *Musa acuminata* cv. Berangan [33], and *Vitis vinifera* L. [34] using air-lift bioreactor (ALB) culture. The similar method of PTC techniques seem as alternatives method for producing secondary metabolites [35] when natural supply is inadequate or chemical synthesis is impossible [36].

Method of production by PTC has some advantages over natural production and even endorsed by Food and Agriculture Organization [37], i.e. easy and scalable production of secondary metabolites [38], rapidity of production[30], independent of season [27], fully controllable room during process [39], produce chemicals in environmentally safely manner [36], uniformity and standardization of the desired compounds [40], and independent of climatic and/or any various environmental factors [41].

Large-scale liquid cultures have been used for micropropagation through organogenesis [28] or somatic embryogenesis pathways [31]. Flask culture and bioreactor have been employed mainly for cell suspension cultures and secondary metabolites production [42] such as in *Arnebia* sp. [43]. Cell suspension culture is most amenable to large-scale production of cell in term of rapid cell proliferation and large number [44]. The objectives of the present study were to determine the best initial cells density (ICD) in flask and ALB cultures for mass cells proliferation and to determine the limonin content in cells.

MATERIALS AND METHODS

The studies were conducted in Tissue Culture Laboratory of Indonesian Citrus and Subtropical Fruits Research Institute (ICSFRI or Balitjestro). The pro-embryonic masses were initiated in 2015 and cell proliferations in flask and bioreactor cultures were conducted in 2016. Citrus limonin and metabolites were analyzed from cells derived optimized protocols [10, 45].

Culture Establishment

Young fruits were collected from mother plant of *C. limonia* cv. JC planted in the experimental field in ICSFRI. Fruits were then washed under running tap water and transferred to laminar air-flow cabinet (LAFC). A sequential sterilization process was subjected to the explants for cleaning the surface following as described previously [31]. Selected normal young seeds were planted on MS basal medium (Duchefa M220) supplemented with 0.5 g/l malt extract (ME), 30 g/l sucrose, 13% (w/v) agar, and 1 mg/l BAP to induce embryogenic calli. The pH of the medium was adjusted to 5.7±0.1 with 0.5 M NaOH before autoclaving at 121°C and 15 psi (103 kPa) for 15 min. The embryogenic calli were sub-cultured biweekly on fresh semisolid medium for selecting homogenous cell growth and habituating pro-embryonic masses (PEM). After 10 subcultures, the PEM were then transferred to liquid culture for another cell adaptation. Cell suspension initiation was conducted as Agisimanto *et al*. [46]. Cultures were sub-cultured biweekly in flasks by removing liquid media and addition of 25 ml fresh liquid medium that similar to medium initiation without agar.

Flask and Air-Lift Bioreactor Cultures

Experiments of flask culture were conducted to evaluate kinetic growth of cells. Cells at 2.0, 4.0, and 6.0 mg/ml were inoculated to 25 ml liquid MS medium supplemented with 0.5 mg/l BAP and 500 mg/l ME. The quantities of explants were equal to 50, 100, and 150 mg PEM per 25 ml medium. The cultures were mechanically agitated on a rotary shaker at 100 rpm at 25°C. The liquid media were refreshed biweekly for six times. ALB cultures were conducted after establishing suitable growth of cells in flask cultures for cell proliferation. Experiments on ALBs to verify the best condition for scaling-up were conducted. Explants at similar density to that in flask cultures were inoculated into 3 l ALBs containing 1 l MS basic medium supplemented with 0.5 mg/l BAP and 500 mg/l ME. The quantities of explants were equal to 2, 4, and 6 mg/ml in 2 l medium. The cultures were supplemented with continuous aeration at 3 vvm. Air was pumped into the ALB via a 0.22 µm filter. A flowmeter was placed between the air filter and pump on each culture to control the rate of aeration. The liquid media were refreshed monthly during 3 months of culture. After 30 days of initiation, liquid media were increased to 2 l for maintaining cells suspension. All the cultures were incubated at 23±2°C under continuous illumination with cool white fluorescent lamps with light intensity at 35 µmol/m^2/s.

Experimental Setup, Data Collection, and Statistical Analysis

In order to compare the kinetic growth of JC in flask and ALB cultures, two separate experiments were carried out under a completely randomized design with three replications per treatment. About 162 flasks containing 25 ml medium were inoculated with cells at three level of ICD (2, 4, and 6 mg/ml), repeat 3 times, 54 units of ALB containing 1,000 ml medium were also inoculated at similar ICD as flask cultures mentioned above. After 15, 30, 45, 60, 75, and 90 days of culture, three samples of flask and an ALB culture were collected and dried on filter paper for 2 h biweekly to measure the kinetic growth of cells, expressed as fresh cell (FW) each treatment and dried cells (DW) after kept in a dryer at 50–60ºC for at least 6 h. The biweekly growth rate (GR) of cells was calculated as final weight (W_f)–initial weight (W_i) per time (t).

Data were analyzed individually per set of treatment by analysis of variance, and multiple comparison analysis was made using least significant differences (LSD) using Genstat 19th edition. Probabilities less to or equal to 0.05 were considered significant.

RESULTS AND DISCUSSIONS

Growth and cell proliferation in liquid medium were significantly affected by ICD. Pattern of cell growth represented by FW and DW in flask and ALB cultures increased exponentially following sigmoid curves (Fig. 1). The patterns of sigmoid curves were specific and significantly different in every treatment of ICD, both in flask and ALB cultures. In this study, inoculation of cells at 2, 4, and 6 mg/ml into flask cultures that were refreshed every 15 days, increased the biomasses significantly until day 75 of cultures.

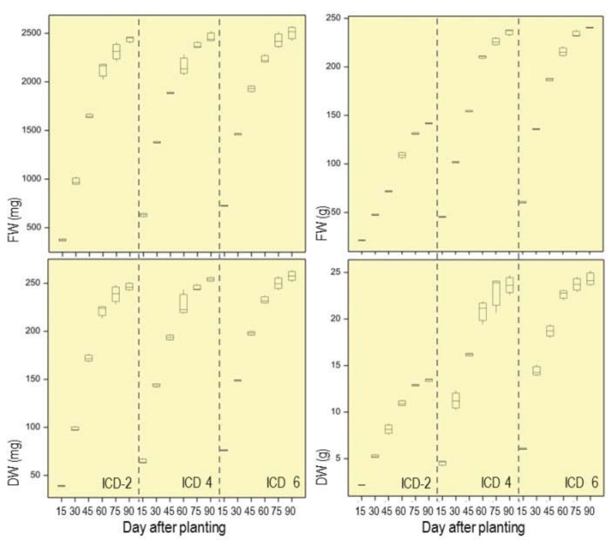

FIGURE 1. Fresh cells weight (FW) and dried weight (DW) of *Citrus limonia* cv. Japanche citroen at ICD-2 (2 mg/ml), ICD-4 (4 mg/ml), and ICD-6 (6 mg/ml) under flask (left) and air-lift bioreactor (ALB) cultures (right) during 90 days of cell suspension culture.

The FW, DW, and GR of both cultures increased linearly at day 15 to 45 after initiation. However, the cells in ALB cultures increased faster in early cycle of culture compared to flask cultures. The GRs of ALB cultures were the highest at day 30 of cultures then reduced linearly (Fig. 2), while cells in flask cultures increased up to 45 days of culture, then the GR reduced by the addition of culture period (Fig. 2). After 75 days of culture, the cell number increased stationary and not significantly based on multiple comparison analysis.

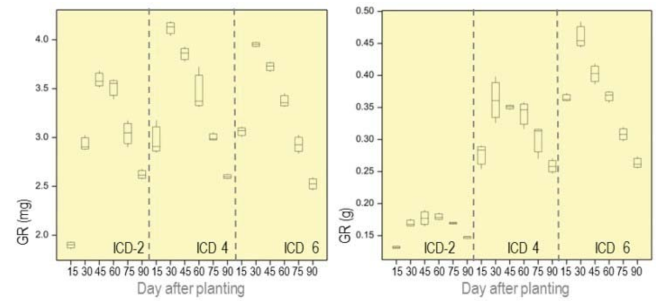

FIGURE 2. Growth rate (GR) of *Citrus limonia* cv. Japanche citroen cells from different at ICD-2 (2 mg/ml), ICD-4 (4 mg/ml), and ICD-6 (6 mg/ml) under flask (left) and air-lift bioreactor condition (right) during 90 days of cell suspension culture.

The total amount of FW and DW of cells inoculated with 2 mg/ml cells increased significantly until 45 days compared to 4 and 6 mg/ml cells, while FW and DW of cells inoculated in flask culture with ICD of 4 and 6 increased only up to 15 days of culture. After 15 days of culture, the cells grew similarly (Tables 1 and 2). Lower concentration of ICD in starting culture produced lower FW and DW at day 90 of culture. However, the GR of 2 mg/ml was still higher until the end of observation (Table 3). The addition of ICD from 2 mg/ml to 4 mg/ml increased the GR from 3.59 mg/day to 3.86 mg/day at 45 day after initiation. Meanwhile, a further increase of ICD from 2 mg/ml to 6 mg/ml of ICD reduced yields by 3.73 mg/day compared to 4 mg/ml of ICD. Based on the DW data at day 45 of culture, cultures inoculated with 2.0 mg ICD/ml multiplied 34-folds while at 4 or 6 mg/ml, the rate of cell multiplication were 19-folds and 13-folds respectively (Table 2).

In ALB cultures, FW, DW, and GR were significantly affected by ICD. In the present study, the total amount of FW and DW of cells biomasses harvested from ALB cultures and GR increased with increasing cells density. The FW at day 30 increased by 25-folds and 22-folds when cell density was increased from 2 to 4 and 6 mg/ml, respectively, whereas at 2 mg/ml of cell density, FW increased by only 23 folds (Table 1). The DW at day 30 of cultures increased similarly to FW (Fig. 1). The GR of cultures inoculated with cells at 2, 4, and 6 mg/ml reached maximum value, i.e. 170, 360, and 400 mg/day, respectively, which is 57, 87, and 116 times higher than those of flask cultures at day 30 (Fig. 2, Table 3). The rapid growth of cell biomasses in ALB cultures compared to flask cultures was significantly different even though the same amount of cells were administered.

Cell growth in ALB was enhanced rapidly with an increase of ICD from 2 to 6 mg/ml. At low ICD (2 mg/ml), the final yield was lower, i.e. 141.7 g at day 90 of culture, compared to 235.8 and 240.4 g of fresh cells at ICD of 4 and 6 mg/ml, respectively. On the contrary, the increase in inoculum size in flask culture was not accompanied by higher cell growth rate. It is also shown that DW at initial density of 4 mg/ml grew faster in the first period of 30 days after inoculation (DAI), while at density 2 and 6 mg/ml grew slower. Even at the lower initial cell inoculums (4 mg/ml) the cells grew faster in the early stage of growth. Nevertheless, the total amount of cells produced in the 6 mg/ml inoculum culture was still higher than that in the other treatments (Table 2). Based on GR parameter, ALB cultures inoculated with 4 mg/ml of ICD reached maximum GR at day 30 of culture, at the rate of 0.36 g/day, while at the same level of ICD, flask culture reached maximum GR of 4.12 mg/day. At ICD of 4 mg/ml, cell growth followed a linier curve during a period of culture at day 15 to 45. This type of growth curve was important to get faster cell growth to reach more than 150 g cells. At ICD of 6 mg/ml, the cells grew slower. This study indicated that lower ICD stimulated faster cell growth in the early stages of culture. However, the total amount of cells produced at the higher inoculum was still higher than at the lower inoculum.

TABLE 1. Inoculum cell density effect on the fresh weight of cells of *Citrus limonia* cv. Japansche citroen in flask and air-lift bioreactor (ALB) cultures.

Inoculum cell density (mg/ml)	Days after planting*					
	15	30	45	60	75	90
Flask			**Cell fresh weight (mg)**			
2	273 a	977 a	1,650 a	2,125 a	2,311 a	2,438 a
4	630 b	1378 b	1,886 b	2,162 ab	2,378 ab	2,461 a
6	726 c	1462 b	1,929 b	2,235 bc	2,427 bc	2,504 b
P-value	<0.01					
CV (%)	3.10					
ALB			**Cell fresh weight (g)**			
2	21.4 a	47.7 a	71.8 a	109.1 a	131.3 a	141.7 a
4	45.5 b	101.8 b	154.5 b	210.1 b	226.0 b	235.8 b
6	60.6 c	135.8 c	187.0 c	215.2 c	234.0 c	240.4 c
P-value	<0.001					
CV (%)	1.60					

*Means in the same column followed by the same letter are not significantly different at P≤0.05 according to the Duncan's Multiple Range Test.

TABLE 2. Inoculum cell density effect on the dry weight of *Citrus limonia* cv. Japansche citroen in flask and air-lift bioreactor (ALB) cultures.

Inoculum cell density (mg/ml)	Days after planting*					
	15	30	45	60	75	90
Flask			**Cell dry weight (mg)**			
2	39 a	98.5 a	172.1 a	221.3 a	238.6 a	246.4 a
4	54.7 b	143.7 b	193.6 b	228.3 ab	245.4 ab	254.2 ab
6	76.1 c	148.8 b	197.9 b	232.7 bc	249.9 bc	257.6 c
P-value	<0.001					
CV (%)	3.00					
ALB			**Cell dry weight (g)**			
2	2.17 a	5.27 a	8.17 a	10.96 a	12.90 a	13.43 a
4	4.54 b	11.24 b	16.18 b	20.82 b	22.89 b	23.64 b
6	6.08 c	14.42 c	18.71 c	22.65 c	23.73 bc	24.30 c
P-value	<0.001					
CV (%)	5.30					

*Means in the same column followed by the same letter are not significantly different at P≤0.05 according to the Duncan's Multiple Range Test.

TABLE 3. Inoculum cell density effect on the growth rate of *Citrus limonia* cv. Japanche citroen on flask and air-lift bioreactor (ALB) cultures.

Inoculum cell density (mg/ml)	Days after planting*					
	15	30	45	60	75	90
Flask			**Cell grow rate (mg/day)**			
2	1.90 a	2.94 a	3.59 a	3.51 c	3.04 b	2.62 a
4	2.98 c	4.12 c	3.86 bc	3.47 ab	3.01 b	2.60 a
6	3.06 c	3.95 b	3.73 b	3.37 a	2.93 a	2.53 a
P-value	<0.01					
CV (%)	3					
ALB			**Cell grow rate (g/day)**			
2	0.13 a	0.17 a	0.18 a	0.18 a	0.17 a	0.15 a
4	0.28 b	0.36 b	0.35 b	0.34 b	0.30 b	0.26 b
6	0.37 a	0.37 b	0.40 c	0.37 b	0.31 b	0.26 b
P-value	<0.001					
CV (%)	5.5					

*Means in the same column followed by the same letter are not significantly different at P≤0.05 according to the Duncan's Multiple Range Test.

At the time of inoculation, a plant cell culture needs a minimum inoculum concentration as the level of nutrient per cell is important for the initial cell division [47]. Cell culture needs a minimum number of cells in the medium at the time of inoculation, which determines the level of nutrition required for each cell for initial cell division. The stimulation of cell growth with increased ICD might result from cell-cell communication among the inoculum cells [48]. The increase in ICD has been shown to achieve higher levels of cell proliferation in other citrus [49] and even more in bioreactor as reported in ginseng [50].

Our study also found that ALB supported rapid and faster cell growth and proliferation and also larger quantity of cells than flask cultures, for several advantages reasons as mentioned earlier [44]. ALB culture provides beneficial for massive production system of healthy organs and the growth of valuable plants throughout the year [51]. In order to obtain the highest yield of production, those factors mentioned above should be determined prior to the application on a bioreactor [52]. The best conditions for cell, organ and/or plant growth and development can be conveniently achieved in a bioreactor by completely regulating several chemical and physical factors, including mixing, gaseous composition, efficient oxygen transfer, pH, and hydrodynamic forces [50].

From the optimized ALB cultures (Fig. 3), the 30 days period of culture produced limonin at 120.725 µg/g DW, while total phenolic was 9.883±0.471 mg GAE/g DW and total flavonoid was 0.144±0.011 mg QE/g DW. These results were lower than the secondary metabolite production from natural leaves that produced limonin at 250.980 µg/g DW, total phenolic at 9.883±0.470 mg GAE/g DW, and total flavonoid at 8.152 ± 0.918 mg QE/g DW.

FIGURE 3. Cell proliferation of *Citrus limonia* cv. Japansche citroen in 100 ml flask at 30 days of culture (left) and in 3 l ALB culture (right).

As bio-process optimization and scale-up of plant cell cultures containing secondary metabolites require an understanding of substrate and inoculums requirements for the production of cell mass, our recent finding confirmed the importance of cell density to produce elite cells in short period of time at high quantity. This information is important for commercial purposes in terms of biomasses and/or secondary metabolites production in bioreactor and amenable lower operational cost compared to natural production. This would be also possible for citrus-based material in Indonesia.

CONCLUSION

Increased cell density affected cell growth in flask and ALB cultures. Flask culture grew slower than ALB culture and produced lower quantity of biomass. The maximum GR in flask culture reached maximum of 4.12 mg/day at day 30 of culture inoculated with 4 mg/ml of ICD. While at the same day, the best GR of cell in ALB culture reached 360 mg/day at cultures inoculated with 4 mg/ml of ICD. ICD at 100 mg per 25 ml medium in flask culture produced 1.378 mg FW cells, 87-folds lower than ALB culture inoculated with 4 g/l medium. From optimized ALB culture, the 30 days period of culture produced limonin of 120.725 µg/g DW, while the total phenolic was 9.883±0.471 mg GAE/g DW and the total flavonoid was 0.144±0.011 mg QE/g DW.

ACKNOWLEDGEMENTS

The authors express gratitude to Evy Anggraeni, Sugeng Sutrisno, Eko Setiawan, and all Flaboratory assistants for excellent lab works. Special thanks to Director and management of ICSFRI for supporting budget of research and Mr. Kaliawan for secondary metabolites analysis.

REFERENCES

1. W. Ahmed and R. Azmat, "Citrus: an ancient fruits of promise for health benefits," in *Citrus-Health Benefits and Production Technology*, edited by M. Sajid and Amanullah (IntechOpen, London, 2019), pp. 19–30.
2. R. Gualdani, M. M. Cavalluzzi, G. Lentini and S. Habtemariam, Molecules **21(11)**, 1530 (2016).
3. G. D. Manners, J. Agric. Food Chem. **55(21)**, 8285–8294 (2007).
4. S. Wang, H. Tu, J. Wan, W. Chen, X. Liu, J. Luo, J. Xu and H. Zhang, Food Chem. **199**, 8–17 (2016).
5. D. Hamdan, M. Z. El-Readi, A. Tahrani, F. Herrmann, D. Kaufmann, N. Farrag, A. El-Shazly and M. Wink, Z. Naturforsch. C J. Biosci. **66(7–8)**, 385–393 (2011).
6. D. Hamdan, M. Z. El-Readi, A. Tahrani, F. Herrmann, D. Kaufmann, N. Farrag, A. El-Shazly and M. Wink, Food Chem. **127(2)**, 394–403 (2011).
7. M. Russo, A. Arigò, M. L. Calabrò, S. Farnetti, L. Mondello and P. Dugo, J. Funct. Foods **20**, 10–19 (2016).
8. M. Jose, T. Sunilkumar and V. Antony, Int. J. Bioassays **3(12)**, 3594–3597 (2014).
9. R. Tundis, M. Loizzo and F. Menichini, Crit. Rev. Food Sci. Nutr. **54**, 225–250 (2014).
10. N. E. Palupi and D. Agisimanto, presented at Temu Nasional Inovasi Pengelolaan, Pemanfaatan, dan Festival Sumberdaya Genetik Lokal, BPTP Jawa Timur, Malang, 2017 (unpublished).
11. J. D. de Souza, E. M. de Andrade Silva, M. A. C. Filho, R. Morillon, D. Bonatto, F. Micheli and A. da Silva Gesteira, PLoS One **12(5)**, e0177993 (2017).
12. L. Sandjo and V. Kuete, "Triterpenes and steroids from the medicinal plants of Africa," in *Medicinal Plant Research in Africa*, edited by V. Kuete (Elsevier, London, 2013), pp. 135–202.
13. Y. S. Shi, Y. Zhang, H. T. Lia, C. H. Wu, H. R. El-Seedi, W. K. Ye, Z. W. Wang, C. B. Li, X. F. Zhang and G. Y. Kai, J. Funct. Foods **75**, 104–213 (2020).
14. Y. Yang, X. Wang, Q. Zhu, G. Gong, D. Luo, A. Jiang, L. Yang and Y. Xu, Bioorg. Med. Chem. Lett. **24(7)**, 1851–1855 (2014).
15. K. Langeswaran, S. G. Kumar, S. Perumal, R. Revathy and M. P. Balasubramaniam, Biomed. Prev. Nutr. **3(2)**, 165–171 (2013).
16. J. Kim, G. K. Jayaprakasha and B. S. Patil, Food Funct. **4(2)**, 258–265 (2013).
17. S. Shimizu, S. Miyamoto, G. Fujii, R. Nakanishi, W. Onuma, Y. Ozaki, K. Fujimoto, T. Yano and M. Mutoh, J. Clin. Biochem. Nutr. **57**, 39–43 (2015).
18. K. N. C. Murthy, G. K. Jayaprakasha and B. S. Patil, Food Funct. **4(5)**, 803–810 (2013).
19. F. Hafeez, W. Akram and E. A. S. Shaalan, Parasitol. Res. **109(1)**, 221–229 (2011).
20. D. S. Kelley, Y. C. Adkins, S. J. Zunino, L. R. Woodhouse, E. L. Bonnel, A. P. Breksa, G. D. Manners and B. E. Mackey, J. Funct. Foods **12**, 271–281 (2015).
21. S. Ben-Othman, I. Jõudu and R. Bhat, Molecules **25(3)**, 510 (2020).
22. S. Fan, C. Zhang, T. Luo, J. Wang, Y. Tang, Z. Chen and L. Yu, Molecules **24(20)**, 3679 (2019).
23. S. Vardhan and S. K. Sahoo, arXiv preprint arXiv:2004.08095 (2020).
24. H. Wang, X. Li, T. Li, S. Zhang, L. Wang, X. Wu and J. Liu, Eur. J. Clin. Microbiol. Infect. Dis. 1–7 (2020).
25. S. Vardhan and S. K. Sahoo, Comput. Biol. Med. **124**, 103936 (2020).
26. S. R. Imadi, K. Shazadi, A. Gul and K. R. Hakeem, "Sustainable crop production system," in *Plant, Soil and Microbes: Volume 1: Implications in Crop Science*, edited by K. R. Hakeem, M. S. Akhtar and S. N. A. Abdullah (Springer International Publishing, Cham, 2016), pp. 103–116.
27. R. Eibl, P. Meier, I. Stutz, D. Schildberger, T. Hühn and D. Eibl, Appl. Microbiol. Biotechnol. **102(20)**, 8661–8675 (2018).
28. C. Q. López, P. Corral, B. Lorrain-Lorrette, K. Martinez-Swatson, F. Michoux and H. T. Simonsen, Plant Methods **14(1)**, 79 (2018).
29. S. Deepthi and K. Satheeshkumar, Biochem. Eng. J. **117**, 198–209 (2017).
30. Z. Haida, A. Syahida, S. M. Ariff, M. Maziah and M. Hakiman, Sci. Rep. **9(1)**, 9533 (2019).
31. D. Agisimanto, N. M. Normah and R. Ibrahim, Agrivita **41**, 284–294 (2019).

32. J. F. Yang, X. C. Piao, D. Sun and M. L. Lian, Sci. Hort. **125(4)**, 712–717 (2010).
33. W. Y. W. Chin, M. S. M. Annuar, B. C. Tan and N. Khalid, Sci. Hort. **172**, 39–46 (2014).
34. M. Y. Jin, X. C. Piao, J. R. Xiu, S. Y. Park and M. L. Lian, Sci. Hort. **164**, 35–40 (2013).
35. H. Gajula, K. Vadlapudi, P. D. Vijendra, J. Rajashekar, T. Sannabommaji, G. Basappa and T. U. Santhosh, "An alternative approach for anticancer compounds production through plant tissue culture techniques," in *Anticancer Plants: Natural Products and Biotechnological Implements: Volume 2*, edited by M. S. Akhtar and M. K. Swamy (Springer Singapore, Singapore, 2018), pp. 529–549.
36. S. Gonçalves and A. Romano, *Secondary Metabolites, Sources and Applications* (IntechOpen, London, 2018).
37. M. I. Dias, M. J. Sousa, R. C. Alves and I. C. F. R. Ferreira, Ind. Crops Prod. **82**, 9–22 (2016).
38. T. Efferth, Engineering **5(1)**, 50–59 (2019).
39. S. Bhatia, "History and scope of plant biotechnology," in *Modern Applications of Plant Biotechnology in Pharmaceutical Sciences*, edited by S. Bhatia, K. Sharma, R. Dahiya and T. Bera (Academic Press, Boston, 2015), pp. 1–30.
40. L. Y. Solís-Ramos, L. M. Carballo and M. Valdez-Melara, Rev. Biol. Trop. **61**, 1095–1107 (2013).
41. S. Bhatia and T. Bera, "Classical and nonclassical techniques for secondary metabolite production in plant cell culture," in *Modern Applications of Plant Biotechnology in Pharmaceutical Sciences*, edited by S. Bhatia, K. Sharma, R. Dahiya and T. Bera (Academic Press, Boston, 2015), pp. 231–291.
42. T. Isah, S. Umar, A. Mujib, M. P. Sharma, P. E. Rajasekharan, N. Zafar and A. Frukh, Plant Cell Tissue Organ Cult. **132(2)**, 239–265 (2018).
43. K. Gupta, S. Garg, J. Singh and M. Kumar, 3 Biotech. **4(3)**, 263–273 (2014).
44. R. B. Santos, R. Abranches, R. Fischer, M. Sack and T. Holland, Front. Plant Sci. **7**, 297–297 (2016).
45. T. Asai, T. Matsukawa and S. i. Kajiyama, J. Biosci. Bioeng. **123(3)**, 376–381 (2017).
46. D. Agisimanto, M. N. Normah, R. Ibrahim and A. Mohamad, Afr. J. Biotechnol. **11**, 2879–2888. (2012).
47. D. Sinlaparaya, P. Duanghaklang and S. Panichajakul, Shengwu Gongcheng Xuebao/Chin. J. Biotechnol. **23(6)**, 1033–1036 (2007).
48. C. O. Akalezi, S. Liu, Q. S. Li, J. T. Yu and J. J. Zhong, Process Biochem. **34(6)**, 639–642 (1999).
49. N. Februyani, W. Widoretno and S. Indriyani, J. Exp. Life Sci. **6(1)**, 1–4 (2016).
50. N. T. Thanh, H. N. Murthy and K. Y. Paek, Ind. Crops Prod. **60**, 343–348 (2014).
51. H. Y. Cui, H. N. Murthy, S. H. Moh, Y. Y. Cui, E. J. Lee and K. Y. Paek, Ind. Crops Prod. **53**, 28–33 (2014).
52. P. Nartop, "Engineering of biomass accumulation and secondary metabolite production in plant cell and tissue cultures," in *Plant Metabolites and Regulation Under Environmental Stress*, edited by P. Ahmad, M. A. Ahanger, V. P. Singh, D. K. Tripathi, P. Alam and M. N. Alyemeni (Academic Press, Boston, 2018), pp. 169–194.