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

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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

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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 24th-25th 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

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AIP Conf. Proc. 2462, 010001-1–010001-1; https://doi.org/10.1063/12.0006897
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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

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

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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

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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

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-
  - PDF
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  - ADD TO FAVORITES
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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

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-
  - PDF
  - E-READER
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  - SHARE
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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

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-
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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

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-
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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

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-
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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

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

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

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

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-
  - PDF
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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
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-
  - PDF
  - E-READER
  - ADD TO FAVORITES
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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

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  - PDF
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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

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-
  - PDF
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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

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-
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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

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-
  - PDF
  - E-READER
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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

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-
  - PDF
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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

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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

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  - PDF
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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

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  - PDF
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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

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Identifying potential seedless citrus accessions through floral structure and pollen performance

Baiq Dina Mariana, Anis Andrini and Sri Andayani
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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

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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

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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

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-
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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

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-
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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

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-
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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

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-
  - 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

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

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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

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-
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  - ADD TO FAVORITES
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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

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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

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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

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-
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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

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-
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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
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- SHARE
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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

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

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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

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-
  - PDF
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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

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-
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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

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

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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

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-
  - PDF
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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

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

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AIP Conference Proceedings **2462**, 030003 (2022); https://doi.org/10.1063/5.0075665

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-
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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

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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

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

Tasliah, Kurniawan Rudi Trijatmiko and Joko Prasetiyono
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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
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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
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Cytological and molecular identifications of seedless tangerine derived from endosperm culture

Chaireni Martasari, Mia Kosmiatin, Ali Husni, Kurniawan Budiarto and Innez Candri Gilang Purnama
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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

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

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

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

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

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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

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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

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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

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-
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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

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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

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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

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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

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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

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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

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-
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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

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

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

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

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-
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  - ADD TO FAVORITES
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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

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-
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  - ADD TO FAVORITES
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  - 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

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-
  - PDF
  - E-READER
  - ADD TO FAVORITES
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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

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-
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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

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-
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  - ADD TO FAVORITES
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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

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-
  - PDF
  - E-READER
  - ADD TO FAVORITES
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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

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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

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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

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  - ADD TO FAVORITES
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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

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  - SHARE
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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

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-
  - PDF
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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

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

Cite as: AIP Conference Proceedings **2462**, 020016 (2022); https://doi.org/10.1063/5.0075204
Published Online: 19 January 2022

Chaerani, Try Zulchi P. Hariyadi and Nurwita Dewi

Plant Parasitic Nematodes Infesting Three Minor Legumes (Velvet Bean, Lablab Bean, and Jack Bean)

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Abstract. Once introduced into rhizosphere, plant parasitic nematodes are difficult to control because they are persistent in soil by surviving on alternative hosts. Several minor legume species are suppressive to plant parasitic nematode population. This study was aimed at observing the plant parasitic nematode fauna infesting three minor legumes, velvet bean (*Mucuna pruriens*), lablab bean (*Lablab purpureus*), and jack bean (*Canavalia ensiformis*) in a field experiment. Soil samples were collected from plots of selected accessions planted in rainfed field at Cikeumeuh Experiment Station, Bogor on six sampling dates from August 2019 until March 2020. Microscopic analysis of extracted soil found two ectoparasitic and three endopatasitic nematodes infesting the legume rhizosphere. From the highest to the least abundant number, these were *Criconemella*, *Helicotylenchus*, *Rotylenchulus*, *Pratylenchus*, and *Meloidogyne*. At the beginning of the planting season the number of nematode ranged from 0 to 57 per 200 ml soil which then fluctuated during the crop growing season. Slight differences in nematode population densities among accessions within a legume species were observed. Jack bean supported the least number of nematode, followed by lablab bean and velvet bean. Further studies under controlled environment are necessary to assess nematode multiplication on potential legume accessions with reducing effect on nematode population.

INTRODUCTION

Plant parasitic nematodes are microscopic (0.3 to 5 mm long), nonsegmented worms belonging to the Kingdom Animalia and Phylum Nematoda [1, 2]. They mainly feed on living plant, obtaining their food from roots with stylets [1]. Their feeding activity on root causes nonspecific symptoms on the above ground plant part such as stunting, yellowing, and wilting, which are similar to the symptoms of water and nutrient deficiency [2]. Yield losses of various crops due to plant parasitic nematode infestation were estimated from 17% to 95% [3–6]. Although global damage caused by plant parasitic nematodes has been estimated at US$ 80 billion per year [7], they are underestimated as crop yield reducer because of the atypical disease symptoms they cause [8,9]. Consequently, they may not be properly managed, allowing population increase which eventually can damage crops [10]. Nematode feeding activity on root can also provide an entry point for secondary infection by other organisms, such as bacteria and fungi, and synergistically intensify disease symptom [1].

The nematode life cycle on average is 30 days [2]. Their numbers are low at the beginning of the growing season, but its population can rapidly increase on susceptible crops and can become harmful in a relatively short period of time [2]. Once introduced into a field, nematode will persist in soil and cannot be eradicated because they can survive on alternative hosts [2]. Therefore, population must be reduced to prevent the build-up of nematodes on crop residues and in the soil by destroying infected plants after harvest, fallow, planting cover crops, or crop rotation with resistant varieties or nonhost crop [1, 2].

Minor legumes are less known, less exploited, and considered underutilized [11]. Examples in this category include velvet bean (*Mucuna* spp.), lablab bean (*Lablab* spp.), *Canavalia* spp., *Sesbania* spp., *Phaseoulus* spp., etc. [11]. The comparable quantities of essential amino acids, protein, carbohydrates, fiber, polyunsaturated fatty acid, essential minerals (iron and zinc) and vitamins to that of soybean, can make important contribution to the dietary

The Second International Conference on Genetic Resources and Biotechnology
AIP Conf. Proc. 2462, 020016-1–020016-7; https://doi.org/10.1063/5.0075204
Published by AIP Publishing. 978-0-7354-4172-9/$30.00

requirement of rural inhabitants [12, 13]. Minor legumes could also be exploited as medicine because they contain bioactive substances; as fodder for livestock; as cover crops and green manure [11–14]. They can be cultivated under low input and once established in the field they are generally adaptable to adverse environmental condition and can thrive under extreme stress condition, such as drought, high salinity, acid soil, and waterlogged soil, perhaps because of deep root system [12–14]. Some species are also shade tolerant [12–14]. Some of these legumes have better capacity of nitrogen fixation than soybean [12, 14]. Minor legumes generally have no significant pests and diseases [12].

The Indonesian Agency for Agricultural Research and Development-Indonesian Center for Agricultural and Biotechnology and Genetic Resources Research and Development Gene Bank (IAARD-ICABIOGRAD Gene Bank) conserves eight accessions of velvet bean (*M. pruriens*), 16 of lablab bean (*L. purpureus*; syn. *Dolichos lablab* L.), and six of jack bean (*C. ensiformis*) collected from various regions in Java and Madura. Velvet bean (Indonesia: "koro benguk"), lablab bean or hyacinth bean (Indonesia: "komak"), and jack bean (Indonesia: "koro pedang") are cultivated as leafy vegetables. The leaves, young pods, and immature seeds are consumed after boiled. Because of its relatively high protein content (up to 27%) as compared to soybean (35–39%), the seeds can be used as substitute to soybean, i.e. roasted or fermented to make "tempeh", tofu or other products, or for production of protein rich flour [15, 16]. However, due to toxic hydrogen cyanide (HCN) or cyanogenic glycosides components and anti-nutritional factors which may decrease its digestibility, they must be soaked or prolonged boil to remove these components [13, 14].

Velvet bean, native to Asia, is a climbing legume species that have potential to grow on terrace walls [12]. Velvet bean can be grown as either annual or perennial crop [12]. Lablab bean, a climbing perennial native to Asia, South East Asia or Africa, can be excellent green manure in cereals rotation system [12, 14]. Jack bean is an annual or weak perennial with climbing or bushy form. It is originated from West Indies and Central America [14]. Seeds can be harvested at 9 to 12 months after sowing [17]. These legumes have been reported to have negative impact on plant parasitic nematode population when intercropped with pineapple or maize, and give positive impact on the growth and yield of the main crop [18, 19]. In another study, the number of plant parasitic nematodes on potato cropping systems with velvet bean as summer cover crop was decreased as compared to that of cropping system with sorghum-sudangrass, but did not increase potato yields considerably [20].

IAARD-ICABIOGRAD Gene Bank rejuvenated and characterized the three legume species on a regular basis. Because of its potentiality in reducing nematode population in the soil, we were interested in investigating the nematode fauna infesting their rhizosphere. This study was aimed at observing the plant parasitic nematode fauna infesting three minor legumes, velvet bean (*M. pruriens*), lablab bean (*L. purpureus*), and jack bean (*C. ensiformis*) in a field experiment.

MATERIALS AND METHODS

A total of 30 accessions of velvet bean, lablab bean, and jack bean were planted in rainfed soil at Cikeumeuh Experiment Station of ICABIOGRAD at the end of dry season on August 29th, 2019. Maize was the preceding crop in velvet bean and jack bean plots, whereas taro was the preceding crop in the lablab bean plots. The field was ploughed using a hand tractor. Depending on the availability of seed stocks and land, the plot size and the number of replication differed for each legume species. Seeds of velvet bean and lablab bean were sown in plots of 2 m × 5 m at a planting distance of 50 cm × 50 cm, whereas seeds of jack bean in plots of 3 m × 5 m at a planting distance of 50 cm × 75 cm. Each accession was sown in four furrows per plot. For each planting hole there was one seed sown. Plots were separated by 1-m wide spaces. Lablab bean and jack bean accessions were sown in two replications (Fig. 1). Fertilizers (50 kg urea, 100 kg SP36, and 75 kg KCl) were applied once in furrows at planting time. Weeding was done twice at 3 and 7 weeks after sowing (WAS). Insecticides and fungicides sprays were not applied.

Five accessions of velvet bean or jack bean and seven accessions of lablab bean with high yield potential were selected for nematode sampling (Table 1, Fig. 1). Five soil samples were taken from selected plots at a depth of 10–15 cm using a hand trowel on six dates at 28 to 49-day interval started from 8 days after sowing (DAS) until 197 DAS. Soil samples from each plot were pooled, thoroughly mixed and then a 200-ml subsample was drawn and processed in laboratory for nematode extraction using the combination of sieving and centrifugal floatation technique in sugar solution (454 g/l) [21]. Nematode genus was identified based on a pictorial key [22] and its number was counted under a stereo dissection microscope. The cumulative population growth of each nematode was calculated using the formula for area under the disease progress curve (AUDPC) as described in Chaerani *et al*. [23]:

$$\sum([P_{i+1} + P_i]/2) \times (t_{i+1} - t_i)$$

Where P_i is the nematode population density at the i^{th} observation, t_i is DAS at the i^{th} observation. This formula provides better assessment of disease magnitude than one time assessment because it integrates the host, pathogen, and environmental effects [24].

TABLE 1. List of minor legumes evaluated for plant parasitic nematode fauna and population.

Field code	Accession no.	Name	Flower and stem color
		Velvet bean	
B2	05035-00002	Lok loreng Purwokerto	Purple/green
B4	05035-00004	Lok Purworejo	White/green
B6	05035-00006	Lok Wonogiri	White/green
B10	05035-00009	Lokal Madura	Purple/green
B12	05035-00001	Lokal Purwokerto	Purple/green
B14	05035-00007	Lokal Pasuruan	Purple/green
		Lablab bean	
K2	05033-00001	DL Lokal	Purple/green with purple pigmentation
K3	05033-00002	DL Lokal A	Purple/green with purple pigmentation
K7	05033-00005	DL 10B	Purple/green with purple pigmentation
K8	05033-00008	DL 10	Purple/green with purple pigmentation
K9	05033-00006	DL 12A	Purple/green with purple pigmentation
K10	05033-00010	DL 12B	Purple/green with purple pigmentation
K11	05033-00011	DL 37	Purple/green with purple pigmentation
		Jack bean	
P1	05036-00001	Pedang A	Purplish/green
P3	05036-00003	Lokal Wonosari	Purplish/green
P5	05036-00005	C7	Purplish/green
P7	05036-00007	Lokal Plumbon	Purplish/green
P9	05036-00005	C7	Purplish/green

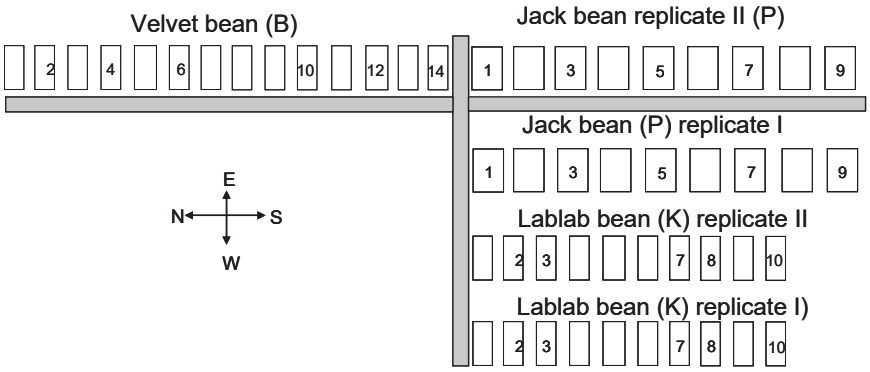

FIGURE 1. Plots of velvet bean (*Mucuna pruriens*), lablab bean (*Lablab purpurea*), and jack bean (*Canavalia ensiformis*) sown in rainfed soil at Cikeumeuh Experiment Station, Bogor for rejuvenation. Figures indicate accessions sampled for soil nematode population investigation.

RESULTS AND DISCUSSIONS

Five nematode genera infesting the three minor legume fields were ring nematode *Criconemella*, spiral nematode *Helicotylenchus*, root knot nematode *Meloidogyne*, lesion nematode *Pratylenchus*, and reniform nematode *Rotylenchulus* (Fig. 2). *Criconemella* and *Helicotylenchus* are ectoparasites, i.e. feed on the peripheral cells or the apex and move freely from one root to another, *Pratylenchus* is a migratory endoparasite, which enters and moves within the root and can leave it to enter another one, whereas *Rotylenchulus* and *Meloidogyne* are sedentary endoparasite which enter and develop within the roots with the female's body becomes swell and sessile within roots [8]. The second stage juveniles of these sedentary nematodes spend short period in the soil before penetrating roots [8]. These nematodes were commonly found in agricultural soil [25, 26] and can survive on weeds [27]. *Meloidogyne* is globally distributed with 98 species described, infects about 2,000 host plant species which makes it the most economically important nematode [7, 26].

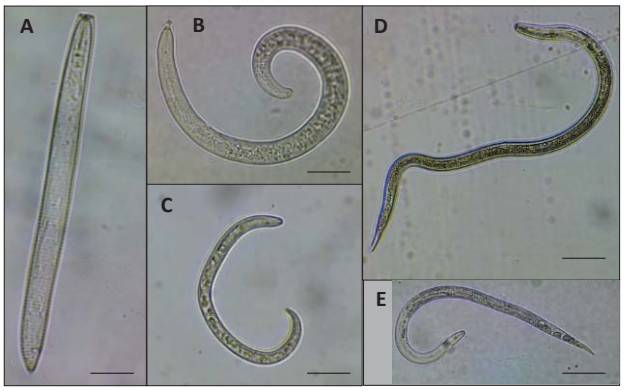

FIGURE 2. Plant parasitic nematodes detected in rhizospheres of velvet bean, lablab bean, and jack bean planted at Cikeumeuh Experiment Station, Bogor: ring nematode *Criconemella* sp. (a), spiral nematode *Helicotylenchus* sp. (b), reniform *Rotylenchulus* sp. (c), lesion nematode *Pratylenchus* sp. (d), and root-knot nematode *Meloidogyne* sp. (e). Bars = 50 µm.

At the first date of sampling (8 DAS) their numbers were undetected or rather low in the rhizosphere, i.e. 1 to 57 (*Criconemella*), 0 to 22 (*Helicotylenchus*), 0 (*Meloidogyne*), 0 to 3 (*Pratylenchus*), and 0 to 10 (*Rotylenchulus*) nematodes per 200 ml of soil (Fig. 3). Although fluctuated during the crop growing season, their numbers were maintained low at the final date of observation (197 DAS), i.e. 0 to 44, 0 to 54, 0 to 1, 0 to 1, and 0 to 24 nematodes per 200 ml soil, for the respective genus. *Criconemella* was detected in all legume rhizosphere at a higher level than the other nematodes and its total number at 197 DAS decreased by only 0.1 to 0.4-fold as compared to that at 8 DAS. *Helicotylenchus* population density increased steeply from 102 to 148 DAS in velvet bean and lablab bean rhizosphere. On all legume species, *Rotylenchulus* was detected at a very low in number from the beginning but started to increase at 102 DAS on velvet bean and at 148 DAS on lablab bean. *Pratylenchus* and *Meloidogyne* remained undetected or low in number at all dates of observations with a negligible population increase. For these two endoparasites, root sampling, which was not performed, may be more appropriate than soil sampling. At the end of crop cycle when plants are senescing, *Pratylenchus* tends to migrate out the roots, and therefore a higher population density in soil could be expected for this nematode [10]. However, because nematode sampling was terminated at ca. 6 months after planting, long before the end of crop cycle, we were unsure whether there was an increase in *Pratylenchus* number in soil.

The area under nematode population growth confirms that *Criconemella* was the dominant genus inhabiting the rhizosphere of all legume species, followed by *Helicotylenchus* and *Rotylenchulus* (Fig. 4). The fact that *Criconemella* and *Helicotylenchus* are ectoparasites with all nematode developmental stages are present in soil explains their higher detection in the soil as compared to the remaining nematode genera.

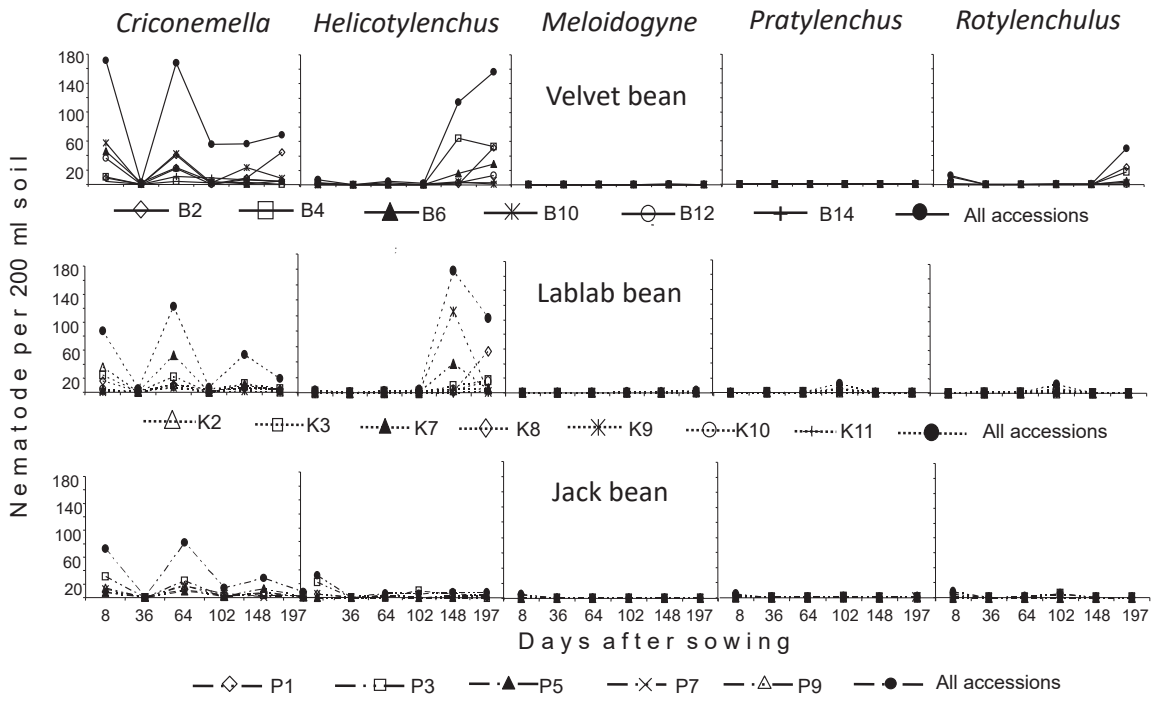

FIGURE 3. Population dynamic of plant parasitic nematode genera in rhizosphere of velvet bean (*Mucuna pruirens*), lablab bean (*Lablab purpurea*), and jack bean (*Canavalia ensiformis*) in rainfed plots at Cikeumeuh Experiment Station, Bogor (from August 2019 until May 2020).

Among accessions within each legume species there were differences in the total nematode population, but the differences on jack bean were in general negligible (Fig. 3). Nematode population in the rhizosphere of lablab bean and jack bean decreased at the final date of sampling. An exception was on lablab bean accession K8, where a 3-fold number of nematode was observed compared to that on 8 DAS. At all dates of observation, the total number of nematode were generally the least in jack bean rhizosphere, whereas that in lablab bean rhizosphere it was intermediate among the three legumes. The area under nematode population growth at 197 DAS further indicates that nematode population densities were the lowest, intermediate, and the highest in jack bean, lablab bean, and velvet bean rhizosphere, respectively (Fig. 4). The total nematode population density in jack bean was 3.0 and 3.6-fold less than that in velvet bean and lablab bean, respectively.

Our finding that the total nematode population was the lowest in the rhizosphere of jack bean corroborates the previous reports [19]. Also, that lablab bean supported an appreciable level of total nematode population is in line with the finding of Osei [18, 28]. In contrast, velvet bean showed nonreducing effect on nematode population, which is not in concordance with many reports [18, 19, 28–32]. Nevertheless, several studies indicated that jack bean or velvet bean did not always have lowering effect on nematode population [10, 20, 33], which may be attributed to different accessions investigated. Another explanation is that a specific host-nematode interaction may operate. For example, velvet bean was a nonhost for *B. longicaudatus*, but was a host for *P. minor* and *Mesocriconema* sp. [20]. A greenhouse study by Arim [19] demonstrated that jack bean and velvet bean were nonhost and lablab bean were poor host to *P. zeae*.

The suppressive effect of jack bean or velvet bean on nematode population could be directly attributed to poor host plant [29], the production of toxic compounds with nematicidal effect that interfere with egg hatching, the nematode's capacity to invade and damage the plants, and reproduce [10, 19], or indirectly by providing a niche to antagonists that inhibit nematodes [34]. *In vitro* assays by Osei [28] demonstrated that leaf and root extracts and also root exudates of velvet bean inhibit *M. incognita* egg hatching and mobility of the hatched juveniles. Indirectly, minor legumes reduced nematode population by enhancing the growth nematode-antagonistic microorganisms. A higher rate of bacteria with nematode-antagonistic properties or chitinolytic fungi suppressive to *Heterodera glycines* and *M. incognita* were isolated from the rhizosphere of velvet bean, castor, jack bean, and rye planted as cover crops as compared to soybean [34, 35].

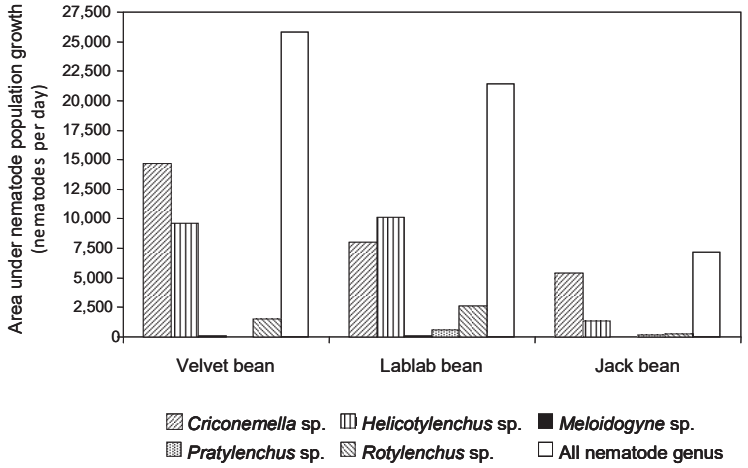

FIGURE 4. Area under nematode population growth at 197 days after sowing in rhizosphere of velvet bean (*Mucuna pruirens*), lablab bean (*Lablab purpurea*), and jack bean (*Canavalia ensiformis*) in rainfed plots at Cikeumeuh Experiment Station, Bogor (from August 2019 until May 2020).

Plant parasitic nematodes are a long-term pest, which are difficult to eradicate once they establish in a site [2]. Proper management to keep their populations low by using minor legumes as cover crops or antagonistic crop in intercropping systems, as nonhost or poor host in crop rotation schemes is environmentally accepted, simultaneously improves crop yield, and cost effective, properties that fit for resource poor farmers in developing countries [10, 18, 28]. Among the three legume species, jack bean is the most potential to suppress nematode population in the field. Jack bean takes up more P from the soil than velvet bean and the low phosphorous condition favors a decrease in *P. zeae* [19]. Jack bean also fixes relatively more N compared with velvet bean, a phenomenon that could have enhanced plant resistance to nematodes through improved growth vigor [19]. In general, jack bean has been reported to be pest resistant, perhaps because it produces HCN and other toxic properties [12]. To select the best accession with suppressive effect on nematode's multiplication, further investigation under controlled environment is needed.

CONCLUSION

Five plant parasitic nematodes infested the rhizosphere of velvet bean, lablab bean, and jack bean at a rate of 0 to 57 nematodes per 200 ml at the beginning of observation (8 DAS) and fluctuated during the crop growing season. Depending on nematode genus, the total nematode population increased by 0 to 32 folds at the final date of observation (197 DAS). *Criconemella* predominated nematode's fauna, followed by *Helicotylenchus*, *Rotylenchulus*, *Pratylenchus*, and *Meloidogyne*. Slight differences in nematode population densities were noted among accessions of velvet bean and lablab, but it was negligible on jack bean. The total nematode population density in jack bean rhizosphere was the least as compared to that in lablab bean and velvet bean rhizospheres, indicating the potential of jack bean to suppress soil nematode population.

ACKNOWLEDGEMENTS

The assistances of Mr. Zulkifli in collecting soil samples and Mr. Ade Achmad in processing soil samples are greatly acknowledged. Financial support was partially provided through DIPA ICABIOGRAD 2019.

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