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

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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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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
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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
AIP Conference Proceedings **2462**, 030009 (2022); https://doi.org/10.1063/5.0076395

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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
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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
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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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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

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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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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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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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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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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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-
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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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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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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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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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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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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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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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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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Alberta Dinar Ambarwati, Eny Ida Riyanti, Edy Listanto, et al.

Environmental Safety Assessment of Genetically Engineered Potato Resistant to Late Blight Caused by *Phytophthora infestans*

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Abstract. Development of potato resistant to late blight caused by *Phytophthora infestans* was carried out by inserting *RB* genes from wild species potato, *Solanum bulbocastanum*. The genetically engineered (GE) potato Katahdin SP951 and the hybrid of Atlantic or Granola with Katahdin SP951 have been assessed by the Biosafety Technical Team (BTT) for environmental safety information that included genetic stability, impact on nontarget organisms (other pests and diseases, beneficial soil microorganisms), substantial equivalent on phenotypic characters, and gene flow. The presence of *RB* gene in four different clonal generations showed stable integration of the gene into the plant genome. Experiment on the effect of the GE potato on pests and other diseases in four confined field trial (CFT) locations showed that the GE potato did not affect to *Alternaria solani* and *Liriomyza* as well as other pests found at CFT including potato tuber moth (PTM), aphids, and mites. Research on the effect of GE potato on beneficial soil microorganisms (nitrogen fixing bacteria, phosphate solubilizing bacteria, and soil fungus) showed that GE potato did not affect the microbial population. The GE potato also had no effect on C/N value when compared to non-GE potato. Agronomic equivalence of the GE potato to conventional comparison (non-GE potato) at biosafety containment and CFT showed that the GE Katahdin SP951 is equivalent to non-GE Katahdin and has no potential as a weed. In addition, agronomic equivalence from other studies gave similar results. Information related to the potential for gene transfer was obtained from the data at CFT Lembang and literature studies, and it can be concluded that there was no potential of gene transfer from GE potato at a distance over 10 m. Based on these results, the GE potato was declared environmentally safe by the BTT. The GE potato Katahdin SP951 has obtained environmental safety certificate from the Indonesian Minister of Environment and Forestry.

INTRODUCTION

Potatoes (*Solanum tuberosum* L.) are one of the elite horticultural commodities with the obstacles of pests and diseases during cultivation. One of the main diseases is late blight caused by the pathogenic fungus *Phytophthora infestans*. In Indonesia, the yield loss caused by *P. infestans* can reach 100% if the weather conditions are very conducive to the development of the disease [1]. Development of potato using resistance gene is one strategy which is effective and environmentally friendly in controlling late blight.

There are some gene sources for resistance to late blight in wild species of potato, including *S. bulbocastanum* [2]. This species is a diploid on the basic number of x = 12, self-incompatible species native to Central America. *S. bulbocastanum* resistance sources have not been widely exploited mainly due to crossing barriers with cultivated potato [3].

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Katahdin is a potato cultivar which was bred by the USDA from the cross (USDA 24642 × USDA 40568) in Presque Isle, Maine (USA) in 1923 [4]. This cultivar was licensed in Canada by 1932 with license no. of P-13. Katahdin is resistant to *Potato virus A* (PVA), moderately resistant to blackleg, net necrosis, Verticillium wilt, PVS, PVX, and PVY. However, Katahdin is susceptible to common scab, Fusarium dry rot, late blight, leaf roll, *Rhizoctonia*, and potato wart [4, 5].

Development of Katahdin to acquire the resistance to late blight disease was carried out by inserting *RB* genes from *S. bulbocastanum*. Katahdin was transformed using *Agrobacterium tumefaciens* LBA4044 containing *RB* gene in plasmid pCL04541, and resulted in the GE Katahdin SP951 [6]. The potatoes were donated to the Indonesian Agency for Agricultural Research and Development through the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) and were used as parents to develop Indonesian potato varieties resistant to late blight.

In Indonesia, the GE Katahdin SP951 was crossed with non-GE Atlantic and Granola, producing several hybrid lines containing *RB* genes. The GE potato Katahdin SP951 dan the hybrids have been evaluated for their resistance to late blight *P. infestans* in confined field trials (CFTs) between 2008–2013 in West Java (Pasir Sarongge, Lembang, Pangalengan, and Garut) and Central Java (Banjarnegara), and showed resistance to *P. infestans*. This resistance could reduce 50% of application of fungicide spraying [7].

Before GE potato Katahdin SP951 and the hybrid lines could be distributed for commercialization, they should be assessed for biosafety including environmental safety. Based on the Indonesian Government Regulation no. 21 of 2005 concerning Biosafety of Genetically Engineered Products, and Minister of Environment Regulation no. 25 of 2012 concerning Guidelines for Preparation of Environmental Risk Analysis Documents for Genetic Engineering Products, the Biosafety Technical Team (BTT) conducted the environmental safety assessment of the GE potato Katahdin SP951 based on information on environmental safety assessment, as outlined below.

ENVIRONMENTAL SAFETY ASSESSMENT

Information on Genetically Engineered Potato

Genetic Information

Katahdin SP951 contains single *R* gene (*RB* gene), which is responsible for resistance to late blight disease caused by *P. infestans*. The *RB* gene is regulated by endogenous promoters and endogenous terminator (poly A terminator). Katahdin SP951 also contains the *nptII* gene with CaMV35S promoter and Ocs3' terminator which is the terminator of the *octopine synthase* gene.

RB gene (2.9 kb) in GE Katahdin SP951 expresses resistance to late blight. The *RB* gene promoter (2.5 kb), as well as the *RB* gene terminator, are endogenous elements isolated from *S. bulbocastanum*. *nptII* gene encodes the aminoglycoside 3'-phosphotransferase (aph[3']-II or NPTII) enzyme that confers resistance to kanamycin as *in vitro* selection agents. This antibiotic has been declared safe by the European Food Safety Authority [8].

Genetic Stability

The results of PCR analysis of four clonal generations of GE potato Katahdin SP951 showed the presence of the *RB* gene, which indicated the stability of the gene. Integration of *RB* gene was indicated by 619 bp fragments found in the native promoter attached to the *RB* gene. The presence of *RB* gene in four different clonal generations (G0, G1, G2, and G3) showed stable integration of the gene into the plant genome (Fig. 1) [9].

Effects of GE Potatoes on Nontarget Organisms

The study of the impact of the GE potato on nontarget organisms, pests and other diseases, was carried out in CFTs in Lembang, Pangalengan, and Garut. The total area of the experiment was 1,500 to 3,000 m^2. The experiment was arranged using a randomized complete block design with three replications. Each potato clone was planted in 3 rows, each row consisted of 10 plants.

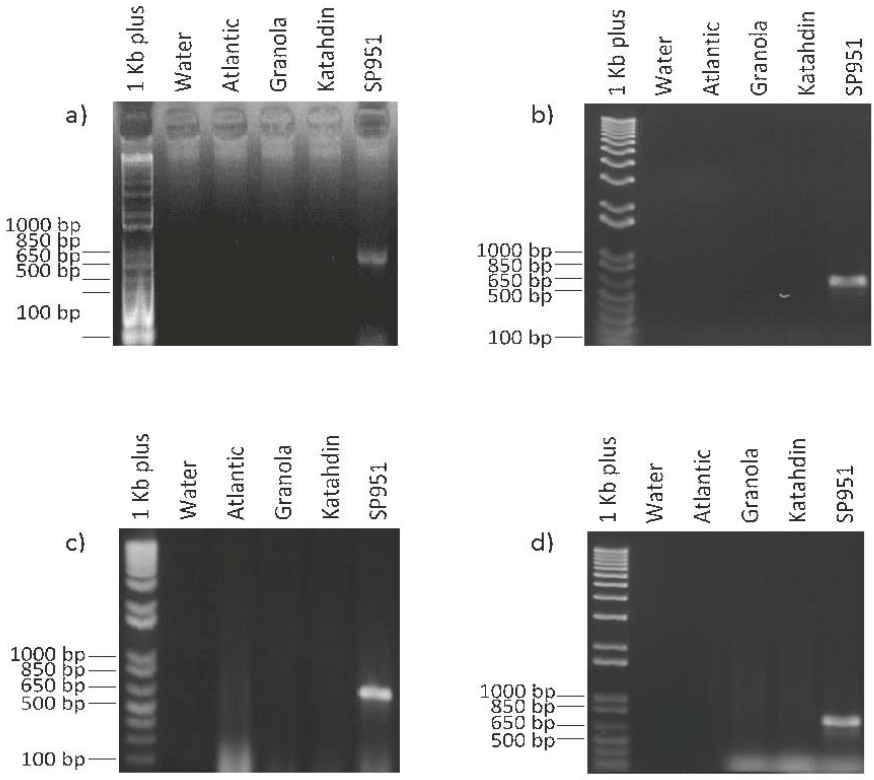

FIGURE 1. Stability analysis of *RB* gene through PCR in four clonal generations (G0, G1, G2, and G3) of Katahdin SP951. (a) G0, (b) G1, (c) G2, and (d) G3. Source: [9].

Effects on Pests and Other Diseases

Experiment on the effect of the GE potato on pests and other diseases was carried out in four CFT locations: Lembang (in 2012), Garut (in 2012), Pangalengan I (Cibunian, Pangalengan, in 2012), and Pangalengan II (Citere, Pangalengan, in 2013). Observations of pests and other diseases were carried out on *Fusarium*, *Alternaria solani*, *Liriomyza*, potato tuber moth (PTM), aphid, and mites, every 1–2 weeks when pests and diseases appear in potato crops. Observation on the number of dead plants due to *Fusarium* did not show any significant difference between Katahdin SP951 and non-GE Katahdin, in each observation times (Table 1).

TABLE 1. The number of dead plants due to *Fusarium* in CFTs Garut (2012) and Pangalengan (2013).

CFT location	Clones	Observation times				
		49 DAP	56 DAP [ns)]	63 DAP [ns)]	70 DAP	77 DAP [ns)]
Garut (2012)	Non-GE Katahdin	0	0	0	0	2.57
	GE Katahdin SP951	0	0	0	0	2.57
Pangalengan (2013)	Non-GE Katahdin	0	0.01	0.04	-	-
	GE Katahdin SP951	0	0.01	0.01	-	-

CFT = confined field trial, GE = genetically engineered, DAP = days after planting, [ns)] = not significant. Source: [10, 11].

The impact of the GE potato on *A. solani* was observed in CFTs Lembang, Pangalengan, and Garut. The results showed that there was no difference in resistance score in GE Katahdin SP951 compared to non-GE Katahdin at each observation times. It indicated that GE potato plants did not have impact to *A. solani*.

TABLE 2. Resistance score to *Alternaria solani* in CFTs Lembang (2012), Pangalengan (2012), and Garut (2012).

CFT location	Clones	Observation times			
		30 DAP [ns)]	36–49 DAP [ns)]	51–56 DAP [ns)]	63–65 DAP [ns)]
Lembang (2012)	Non-GE Katahdin	8.97	8.91	8.22	7.60
	GE Katahdin SP951	8.85	8.88	8.04	7.20
Pangalengan (2012)	Non-GE Katahdin	8.89	8.56	7.33	4.22
	GE Katahdin SP951	8.89	8.56	6.56	5.44
Garut (2012)	Non-GE Katahdin	-	6.55	4.42	4.15
	GE Katahdin SP951	-	6.37	3.32	3.32

CFT = confined field trial, GE = genetically engineered, DAP = days after planting, [ns)] = not significant.

Observation of plants resistance to *Liriomyza* was determined using resistance score based on the percentage of infected leaves [12]. The results showed that resistance in Katahdin SP951 against *Liriomyza* (score 2.07, resistant) was not different from that in the non-GE Katahdin (score 2.32, resistant) at the last observation or 65 DAP (Table 3). It means that planting of GE potatoes showed the same response to non-GE potato on *Liriomyza* attack.

TABLE 3. Resistance score to *Liriomyza* on potato plant in CFT Lembang (2012).

Clones	Observation times			
	30 DAP [ns)]	43 DAP [ns)]	51 DAP [ns)]	65 DAP [ns)]
Non-GE Katahdin	0.32	0.35	0.67	2.32
GE Katahdin SP951	0.43	0.65	1.55	2.07

CFT = confined field trial, GE = genetically engineered, DAP = days after planting, [ns)] = not significant. 1 = very resistant, 2 = resistant, 3 = moderate resistant, 4 = susceptible, 5 = very susceptible [12]. Source: [10, 11].

Observation on pests (PTM and aphids) was determined by counting the number of PTM larvae and aphids, respectively, in each plant. The results showed that there were no significant differences between the average number of PTM larvae in GE Katahdin SP951 (0.63) compared to that of non-GE Katahdin (0.75) at the last observation or 65 DAP (Table 4). The number of aphids per plant also did not show a significant difference between GE Katahdin SP951 (0.75) compared to non-GE Katahdin (1.68) at observations 56–65 DAP. It means that the GE and non-GE potatoes have similar response toward the PTM and aphids.

TABLE 4. Mean values of potato tuber moth larvae in the CFT Lembang (2012).

Clones	Observation times				
	30 DAP [ns)]	36 DAP [ns)]	43 DAP [ns)]	51 DAP [ns)]	65 DAP [ns)]
Non-GE Katahdin	0.27	15.53	0.20	0.52	0.75
GE Katahdin SP951	0.38	16.90	0.37	0.62	0.63

CFT = confined field trial, GE = genetically engineered, DAP = days after planting, [ns)]: not significant. Source: [10, 11].

Lazebnik *et al.* [13] tested the nontarget effects of a late blight-resistant GE potato on aphid *Myzus persicae* in greenhouse and climate room experiments. He reported that aphid fitness varied considerably more between conventional potato varieties than between Désirée and the GM events. Observations on mites showed that during the observation at CFT, mites were not found both in Katahdin SP951 and non-GE Katahdin. The results of these study showed that Katahdin SP951 did not affect to *A. solani* and *Liriomyza*, as well as other pests found in CFT, PTM, aphids, and mites. Both GE and non-GE potato gave similar response toward the other pests and diseases.

Effects on Soil Microorganisms

Research on the effect of GE Katahdin SP951 on soil microorganisms that consisted of nitrogen (N)-fixing and phosphate (P)-solubilizing bacteria was carried out in four CFT locations (Lembang in 2012, Garut in 2012, Pangalengan I [Cibunian, Pangalengan] in 2012, and Pangalengan II [Citere, Pangalengan] in 2013). The material used in the study was soil samples taken from the roots of the GE and non-GE potato from the CFT experiment.

Microbial populations in soil samples were determined using plating method with serial dilutions of 10^{-1}–10^{-11} on selection media for N-fixing and P-solubilizing bacteria. Each dilution from 10^{-5}–10^{-11} was spread on selection media with three replicates. Plates were then incubated at 29°C for 2 weeks. The number of growing colonies were counted every day to determine the number of bacterial populations per gram of soil. The results showed that there was no significant difference between the population of N-fixing and P-solubilizing bacteria in the root area of non-GE potato compared to GE potato in each CFT location at observation of 90 DAP (Table 5 and 6).

TABLE 5. Population of N-fixing bacteria (viable cells/g soil) in CFTs Lembang (2012), Pangalengan (2012 and 2013), and Garut (2012).

CFT location	Soil sample	Observation times	
		0 DAP [ns)]	90 DAP [ns)]
Lembang (2012)	Non-GE Katahdin	9.5×10^8	6.8×10^6
	GE Katahdin SP951	1.1×10^9	7.2×10^6
Pangalengan (2012)	Non-GE Katahdin	5.2×10^9	1.5×10^7
	GE Katahdin SP951	4.9×10^9	1.8×10^7
Garut (2012)	Non-GE Katahdin	6.5×10^9	1.3×10^{11}
	GE Katahdin SP951	2.3×10^{10}	4.0×10^{10}
Pangalengan (2013)	Non-GE Katahdin	2.7×10^6	0.9×10^8
	GE Katahdin SP951	2.2×10^6	0.7×10^8

CFT = confined field trial, GE = genetically engineered, DAP = days after planting, [ns)] = not significant. Source: [10, 11].

TABLE 6. Population of P-solubilizing bacteria (viable cells/g soil) in CFTs Lembang (2012), Pangalengan (2012 and 2013), and Garut (2012).

CFT location	Soil sample	Observation times	
		0 DAP [ns)]	90 DAP [ns)]
Lembang (2012)	Non-GE Katahdin	2.6×10^9	4.1×10^{10}
	GE Katahdin SP951	2.4×10^9	4.5×10^{10}
Pangalengan (2012)	Non-GE Katahdin	1.9×10^9	1.5×10^7
	GE Katahdin SP951	2.2×10^9	1.2×10^7
Garut (2012)	Non-GE Katahdin	3.3×10^{10}	4.5×10^{10}
	GE Katahdin SP951	1.7×10^9	1.6×10^{11}
Pangalengan (2013)	Non-GE Katahdin	1.1×10^8	0.9×10^8
	GE Katahdin SP951	1.1×10^8	0.4×10^8

CFT = confined field trial, GE = genetically engineered, DAP = days after planting, [ns)] = not significant. Source: [10, 11].

Population of soil fungus from the rhizosphere was determined using plating method on potato dextrose agar (PDA) medium. The next stage followed the procedure for counting the population of N-fixing and P-solubilizing bacteria. The results showed that the soil fungus population from the rhizosphere of Katahdin SP951 (0.8×10^4) was not significantly different from the non-GE Katahdin (0.2×10^4) at the last observation (90 DAP) (Table 7).

TABLE 7. Population of soil fungus (viable cells/g soil) from the rhizosphere in CFT Pangalengan (2013).

CFT location	Soil sample	Observation times		
		0 DAP [ns)]	45 DAP [ns)]	90 DAP [ns)]
Pangalengan	Non-GE Katahdin	1.5×10^4	0.9×10^4	0.2×10^4
	GE Katahdin SP951	4.0×10^4	2.0×10^4	0.8×10^4

CFT = confined field trial, GE = genetically engineered, DAP = days after planting, [ns)] = not significant.
Source: [10, 11].

These results showed that GE Katahdin SP951 did not affect the population of beneficial soil microorganisms. The microbial population (P-solubilizing and N-fixing bacteria, and soil fungus) was not significantly different in the GE Katahdin SP951 and non-GE Katahdin.

Carbon (C)/N value was analyzed using soil samples of the GE and non-GE potato. Soil samples were taken three times, i.e. before planting (0 DAP), during plant growing (45 DAP), and after harvest (90 DAP). C content was determined using Walkley and Black method [14], meanwhile N content was determined using a method of KJedalh [15]. The results showed that the C/N values of both Katahdin SP951 and its non-GE counterpart were not significantly different in each location (Table 8) [10, 11].

TABLE 8. The C/N ratio of the soil from CFTs Lembang (2012), Pangalengan (2012 and 2013), and Garut (2012).

CFT location	Soil sample	C/N		
		0 DAP [ns)]	45 DAP [ns)]	90 DAP [ns)]
Lembang (2012)	Non-GE Katahdin	12.67	10.00	10.33
	GE Katahdin SP951	15.33	11.00	10.67
Pangalengan (2012)	Non-GE Katahdin	8.33	8.00	8.33
	GE Katahdin SP951	8.67	8.67	8.67
Garut (2012)	Non-GE Katahdin	8.33	9.00	11.33
	GE Katahdin SP951	7.67	8.00	11.00
Pangalengan (2013)	Non-GE Katahdin	9.67	10.00	11.00
	GE Katahdin SP951	9.00	10.00	12.00

CFT = confined field trial, GE = genetically engineered, DAP = days after planting, [ns)] = not significant.
Source: [10, 11].

Potential as Weeds

Agronomic equivalence between GE potato Katahdin SP951 and its non-GE counterpart was observed in the greenhouse of biosafety containment in ICABIOGRAD, Bogor and in two CFTs (Pangalengan in 2013 and Lembang in 2013–2014). The results showed that there were no significant differences in plant height, length and width of their terminal leaves [16]. Observations in CFTs showed that GE potato Katahdin SP951 had the same qualitative characters (i.e. green triangle-shape stem, green oval-shape leaves, white star-shape flowers, white tuber flesh and skin, and cream oval seeds) as non-GE Katahdin.

Potato plants can only growth in a managed ecosystem, in highland areas with altitudes between 1,000–3,000 m above sea level, in soil types of Andosol, Latosol, Regosol, and Alluvial with pH ranging from 5.0–6.5, monthly rainfall between 200–300 mm, temperature of 15–20°C, and humidity of ±70% [17]. Eastham and Sweet [18] reported that *S. tuberosum* cannot establish as weeds, because *S.tuberosum* demonstrates limited competitive abilities, is not a primary colonizer in unmanaged ecosystems, and seedlings do not tend to compete successfully

against cultivated potato crops. Substantially, the GE potato have shown the same qualitative characters as non-GE potato, and therefore, they cannot survive outside agricultural environments and have no potential as weed.

Potato plants are self-pollinating. Some varieties of potatoes are not produced flower, and when flowers are formed, they are dropped after pollination, so that no berries are found [18, 19, 20, 21]. Potato seeds cannot be spread naturally because the seeds are slimy and sticky and contained in the fruit (berries) [17]. *S. nigrum* (black nightshade) and *S. dulcamara* (woody nightshade) are the member of genus *Solanum*, that are known as weed [18, 22]. However, these two species are not found in Indonesia. From the results of biosafety containment and CFTs, and also from the literature, it can be concluded that the GE Katahdin SP951 is equivalent to non-GE Katahdin and has no potential as a weed.

Potential Gene Transfer

Information on environmental safety related to the potential for gene transfer was obtained from the data at CFT Lembang and literature studies. As a self-pollinating plant, the potential for cross-pollination is very small. The estimated rates of cross-pollination under field conditions range from zero to about 20% [18, 22]. Gene transfer can occur through biotic factors such as pollinators or abiotics factors such as wind. The main pollinators in potato plants are bumblebees (e.g. *Bombus funebris* in Peru and *B. impatiens* in the US) [21, 23].

Research on possible *RB* gene transfer from GE potato to non-GE potato through natural hybridization was conducted at CFT Lembang (2008–2009). The GE Katahdin SP951 was planted in one row in the center of the plot. Meanwhile, non-GE Katahdin were planted in one row at a distance between 0.8 m and 11.2 m, with 0.8 m intervals, from the GE Katahdin plant, on the left and right side of the GE Katahdin plant. The experimental site was surrounded by five rows of corn as a border. During the study, there were no potato crops outside the CFT plot. The result showed that there was a gene transfer from GE potato Katahdin SP951 to non-GE potato. The gene transfer was at a isolation distances of (0.8–1.6 m), (2.4–4 m), and (4.8–6.4 m) were 13.78%, 10.92%, and 3.82%, respectively. At a distance of 7.2–8.0 m there was no (0%) gene flow [24].

McPartlan and Dale [22] observed the direction of wind in the study of gene transfer (*nptII* gene) in the field. He reported that the percentage of plants formed berries and the average number of berries per plant was higher in the north sector in the plot compared to the south, west, and east when the wind direction over the plot was predominantly from the southeast. Where the GE and non-GE potato were in alternated rows with their leaves touching, the frequencies of progeny from the non-GE potato that contained the kanamycin-resistance gene ranged from 23.1–28.8%. At the isolation distance of 3 m and 10 m, the frequency decreased to 2% and 0.017%, respectively. When the distance of plants at up to 20 m, no transgene-containing progeny were observed. Frequency of transgene dispersal by pollen to non-GE potato is limited and very unlikely at distances over 10 m [25]. From the results at CFT and literature studies, it can be concluded that there was no potential of gene transfer from GE potato at a distance over 10 m.

CONCLUSION

The GE potato Katahdin SP951 have been assessed by the BTT for environment safety information which consisted of genetic stability, impact on nontarget organisms (other pests and diseases, beneficial soil microorganisms), potential as weeds, and potential gene transfer. According to all the experiments conducted with the support of other studies, the GE potato Katahdin SP951 was not significant different from the non-GE Katahdin on the aspect evaluated. Based on those data, the assessment by BTT declared that GE potato Katahdin SP951 is environmentally safe. The GE potato Katahdin SP951 has obtained environmental safety certificate from the Indonesian Minister of Environment and Forestry in 2018.

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