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
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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FreeJanuary 2022

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

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

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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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  - ADD TO FAVORITES
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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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-
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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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-
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  - ADD TO FAVORITES
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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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  - E-READER
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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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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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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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-
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  - E-READER
  - ADD TO FAVORITES
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FreeJanuary 2022

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

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

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

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M. Muchlish Adie, Titik Sundari, Kurnia Paramita Sari, et al.

Identification of Soybean Promising Lines Resistant to Pod-Sucking Bug, *Riptortus linearis* (Fabricius)

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Abstract. Pod-sucking bug *Riptortus linearis* (Fabricius) is the most destructive soybean insect pest in Indonesia. The losses caused by this insect could reach 2–100%. This study aimed to determine the resistance level of 13 soybean lines to pod-sucking pests using no choice and free choice tests. The evaluation for resistance to the pod-sucking bug was performed using free-choice test (FCT) and no-choice test (NCT) methods. In FCT, all genotypes were placed in a meshed cage, meanwhile, in the NCT, each soybean genotype was individually placed in a meshed cage. Newly emerged adults *R. linearis* (n = 2 per plant) were infested during the R5 stage of the soybean. In the FCT, the range of pod damage intensity was 11.03–44.22% (average of 24.68%). Meanwhile, the seed damage intensity was ranged from 10.94–41.14% (average of 20.93%). In the NCT, the range of pod and seed damage intensities was 18.89–47.52% (average of 37.17%) and 13.19–72.38% (average of 37.74%), respectively. One genotype (G4) was identified as resistant based on the pod and seed damage in the FCT and NCT, respectively. Soybean genotypes that were consistently resistant based on the pod and seed damage in the NCT, as well as FCT methods, could potentially be used as gene sources for breeding soybean resistance to pod-sucking bug. The NCT method provides higher damage intensity than that of the FCT method. Thus, the NCT method could be used to screen soybean genotypes for resistance against pod-sucking bug to obtain genotypes with higher resistance level.

INTRODUCTION

Pod-sucking bug *Riptortus linearis* (Fabricius) (Hemiptera: Alydidae), is a pod-feeding insect pest that is currently the most serious soybean pest in Indonesia. The losses caused by this pest are not only quantitative in the form of reduced seed yield, but also qualitative, as they are related to seed vigor. The amount of yield loss caused by pod-sucking pests varies by soybean production center area. In Indonesia, reported losses exceed 70% [1]. In Africa could reach 20–100% [2]. Meanwhile, the losses in India were reported to be 30–40% [3]. The difference in yield loss is due to the susceptibility of the varieties used as well as the stage of pest infestation.

In Indonesia, the two most common pod-sucking pests are *Nezara viridula* and *R. linearis*. However, in recent years, *R. linearis* has been found to dominate various soybean production centers. As reported by Krisnawati and Adie [4] that the damage intensity of pod-sucking bug at 45 days after planting (DAP) in a controlled environment reached 60.24%, while the damage intensities of pod borer and pod worm were 46.08% and 3.85%, respectively. Other countries have also reported changes in the dominance of pod-feeding pests. The effect of two species of pod-sucking bugs in soybean showed that *R. dentipes* caused more pod damage (70%) to the plant than did *N. viridula* (44%) [5]. Another study reported that the southern green stink bug *N. viridula* was once widespread in the Southern Brazil and Southern USA, but its numbers have steadily declined [6]. In Bangladesh, it was also reported that the *R. pedestris* (Fabricius) and *Halyomorpha halys* (Stål.) as the major insect pest of soybean [7].

The amount of yield loss caused by pest infestation is also related to the time of pest attack. A study discovered that stage R4 and R6 of soybean plants were the most vulnerable to the *H. halys* (Hemiptera: Pentatomidae). These two stages represent the full pods and seeds filling process [8]. Another study reported that the soybean's most vulnerable growth stage to pod-sucking bug infestations occurs between the reproductive stages R3 and R5 [5]. The

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best way to reduce soybean yield losses due to pest attack is to provide soybean varieties that are resistant to these pests. The soybean genotype of IAC 100 has been reported to be resistant not only to pod-sucking pests, but also to other pests in soybeans [9, 10]. Identification of resistance in 21 soybean advanced lines obtained G511H/Anj//Anj-2-8 as a resistant line to pod-sucking bug [11]. In cowpea, the identification of resistance gene to pod-sucking bug *Clavigralla tomentosicollism* obtained the IT86D-716 as resistant genotype [12].

The determinants of a genotype's resistance to specific pests are due to chemical factors, morphology, or escape. Several research findings revealed that one of the morphological factors considered to determine resistance to various pests, including the pod-sucking bug, is the character of trichomes [9, 13–15]. This fact indicates that increasing soybean resistance to pod-sucking pests has a chance of success. This study aimed to determine the resistance level of several soybean advanced lines to pod-sucking pests using no choice and free choice tests.

MATERIALS AND METHODS

Genetic Materials and Study Site

The research materials consisted of 13 soybean genotypes, including 10 lines selected from several cross combinations and 3 check cultivars (2 high yielding genotypes and 1 pod-sucking bug resistant genotype) (Table 1). The experiment was conducted in Malang (East Java, Indonesia) from March to June 2018. The insect use for the test was the progeny (F_1) of adults *R. linearis* collected from soybean fields in the Kendalpayak Experimental Station, Malang and reared in the laboratory.

TABLE 1. Genetic materials used in this study.

No.	Code	Soybean genotype	Remark
1	G1	SPLas-11	Pedigree line
2	G2	SPLas-13	Pedigree line
3	G3	SPLas-14	Pedigree line
4	G4	SPLas-19	Pedigree line
5	G5	SPLas-25	Pedigree line
6	G6	SPLas-8	Pedigree line
7	G7	SPLas-10	Pedigree line
8	G8	SPLas-3	Pedigree line
9	G9	SPLas-2	Pedigree line
10	G10	SPLas-1	Pedigree line
11	G11	Demas 1	Check cultivar for high yield
12	G12	Anjasmoro	Check cultivar for high yield
13	G13	G100H	Check cultivar for insect pest resistance

Research Design and Plant Cultivation

The experiment was arranged in a randomized complete block design with 13 soybean genotypes as treatment and each genotype was replicated three times. Each genotype was planted in a plastic pot (18 cm in diameter) containing a mixture of soil and manure in a 4:1 ratio. The planting was done in such a way that all soybean genotypes could flower at the same time. Before sowing, a 5 g/pot NPK fertilizer was applied. To control pests, an insecticide with the active ingredient of cypermethrin was applied 8 DAP, followed by an insecticide with the active ingredient of cyhalothrin at 14, 21, and 28 DAP. The insecticide application is not carried out after 28 DAP.

Resistance Evaluation

The evaluation of soybean genotypes for resistance to pod-sucking bug was done by using two methods, namely free-choice test (FCT) and no-choice test (NCT). In FCT, all genotypes were placed in a meshed cage, meanwhile,

in the NCT, each soybean genotype was individually enclosed in a meshed cage. The FCT test was performed by placing all of the tested genotypes in one meshed cage when the plants were 35 DAP, so that there were three meshed cages as a reflection of three replications. In NCT, each genotype was individually enclosed in a meshed cage (with three replicates for each) with an iron frame (30 cm in diameter and 60 cm in height) when the plants were 35 DAP. On those methods, each cage was infested with a pair of newly emerging adult *R. linearis* (NCT) and as many as one pair of imago/hill/replication (FCT) at 02.00 PM during the R5 stage.

Observations and Data Analysis

The agronomic characters observed included plant height, seed weight per plant, pod numbers per plant, and seed numbers per plant. Soybean pods were harvested after reaching full maturity. Seeds were manually removed from the pods, and then visually examined under a binocular microscope for the number of punctures on seed caused by *R. linearis*. The measurement formulas for the intensity of damaged pod and seed [10] were:

$$Pod\ damage\ (\%) = \frac{\Sigma\ attacked\ pods}{\Sigma\ total\ pods}\ x\ 100\%$$

$$Seed\ damage\ (\%) = \frac{\Sigma\ attacked\ seeds}{\Sigma\ total\ seeds}\ x\ 100\%$$

The grouping of resistance to pod-sucking bug was based on the pod damage and seed damage, as follows: resistant (<21%), moderately resistant (21–40%), moderately susceptible (41–60%), susceptible (61–80%), and highly susceptible (>80%) [16]. Data were subjected to a two-way analysis of variance (ANOVA). The correlation analysis was performed using the Pearson correlation to measure the relationship between agronomic characters with pod damage and seed damage, respectively.

RESULTS AND DISCUSSIONS

Analysis of Variance for Agronomic Characters and the Damage Intensity

The identification of soybean genotype resistance to the pod-sucking bug using a free-choice test (FCT) and no-choice test (NCT) showed that the performance of the morphological agronomic characters (plant height, seed weight per plant, pod numbers per plant, and seed numbers per plant) is not affected by differences in resistance test methods. On the other hand, the intensity of pod damage, seed damage, and the number of punctures on seed differed between FCT and NCT. Significant differences among genotypes were found (Figs. 1–5). There was a significant interaction between resistance test methods and genotype on the characters of pod damage intensity, seed damage intensity, and the number of punctures on the seeds, indicating the difference in the pressure of the damage intensity of pod-sucking bug in FCT and NCT (Table 2).

TABLE 2. Analysis variance for agronomic characters and the damage intensity caused by pod-sucking bug infestation.

Character	Mean square		
	Resistance test method (R)	Genotype (G)	Interaction (R × G)
Plant height (cm)	75.02 ns	818.76**	65.36 ns
Seed weight per plant (g)	0.27 ns	1.50**	1.05 ns
Pod numbers per plant	5.92 ns	28.79**	20.45*
Seed numbers per plant	220.01 ns	180.34*	143.90^{ns}
Pod damage intensity (%)	3560.76**	281.12*	218.76*
Seed damage intensity (%)	3708.06**	661.72*	746.52*
Number of punctures on seed	126.92*	71.61**	52.87*

**Significant at 1% probability level (p<0.01). *Significant at 5% probability level (p<0.05). ns = not significant.

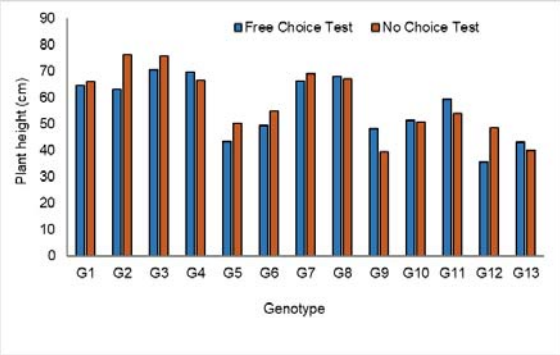

FIGURE 1. Plant height of thirteen soybean genotypes tested in the NCT and FCT.

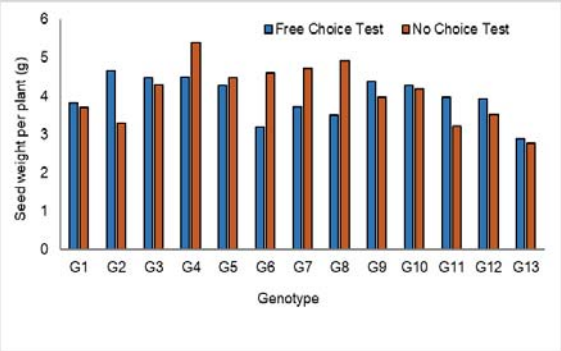

FIGURE 2. Seed weight per plant of thirteen soybean genotypes in the NCT and FCT.

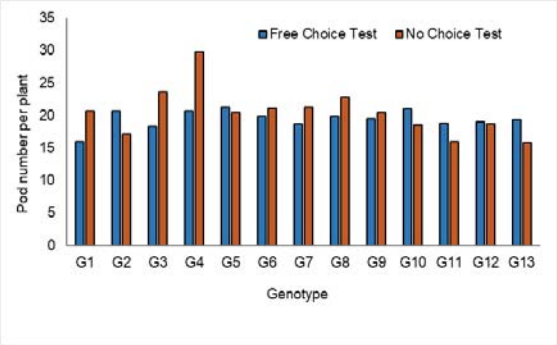

FIGURE 3. Pod numbers per plant of thirteen soybean genotypes in the NCT and FCT.

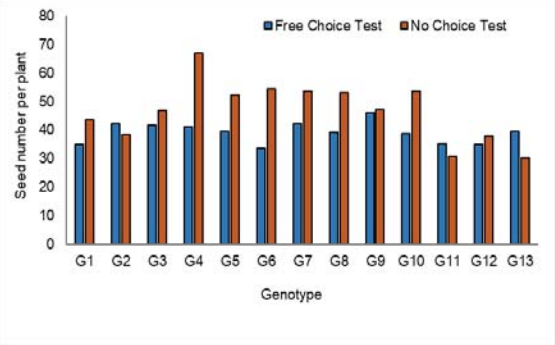

FIGURE 4. Seed numbers per plant of thirteen soybean genotypes in the NCT and FCT.

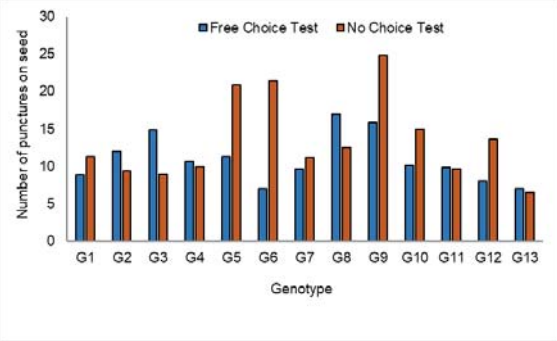

FIGURE 5. Number of punctures on seeds of thirteen soybean genotypes resulted from *Riptortus linearis* infestation in the NCT and FCT.

Resistance Evaluation of Pod-Sucking Bug

Free-Choice Test (FCT)

FCT provides an opportunity for individual insect pests to make host selection. The average intensity of the pod damage and seed damage was 24.68% and 20.93%, respectively. The damaged pod was found to be greater than the seed damage. Based on the intensity of the pod damage, the resistance of 13 soybean genotypes were classified into resistant (4 genotypes), moderately resistant (8 genotypes), and moderately susceptible (1 genotype). Meanwhile, the

resistance of soybean genotypes based on the intensity of the seed damage were classified into resistant (7 genotypes), moderately resistant (5 genotypes), and moderately susceptible (1 genotype).

Two genotypes showing the resistant consistency based on the pod as well as seed damage in the FCT (Fig. 6), while other genotypes have different resistance based on pod and seed damage. This demonstrates that there are different resistance determinants of each soybean genotype due to differences in pod and seed attributes. The morphology and chemical factors were reported to be the soybean determinants of resistance to pod-sucking bug. A study reported that the trichome length and density as the antixenosis resistance in soybean for pod-sucking bug. They also successfully identified two genotypes (IAC 100 and IAC 80) that have a source of resistance gene for breeding [9]. Another study in soybean related to the performance of the resistant genotype (IAC 100) and susceptible genotype (FT-Estrela) to the stink bug-complex suggested using the percentage index of pod damage (PIPD) as selection criteria in the early generation and the percentage of spotted seeds (PSS) for the advanced generation [17].

No-Choice Test (NCT)

The resistance evaluation using the NCT is a force-feeding test because the insect pest was forced to fed the host plant, without other choices of hosts. In this study, the average intensity of pod damage was 38.20%, higher than that of the average intensity of seed damage (34.72%). Based on the intensity of pod damage, 1 genotype was classified as resistant, 6 genotypes as moderately resistant, and the rest (6 genotypes) were moderately susceptible (Fig. 7). The soybean resistance level obtained based on the intensity of seed damage was classified into 3 genotypes as resistant, 6 genotypes as moderately resistant, 3 genotypes as moderately susceptible, and 1 genotype as susceptible. A genotype was obtained to show a consistent resistance to pod-sucking bug based on both the intensity of pod damage as well as seed damage observations.

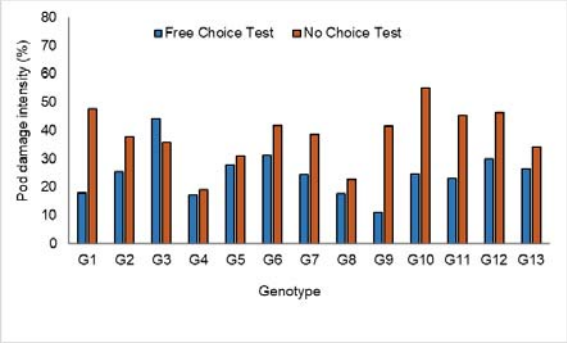

FIGURE 6. Pod damage intensity of 13 soybean genotypes resulted by *Riptortus linearis* infestation in both the NCT and FCT.

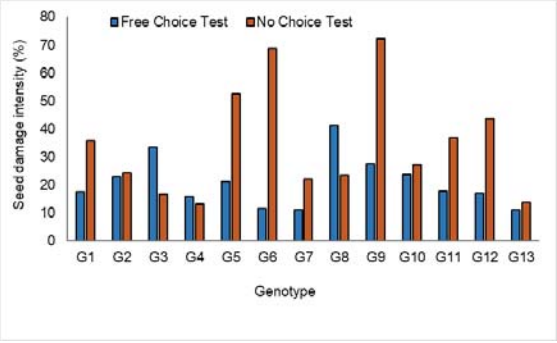

FIGURE 7. Seed damage intensity of 13 soybean genotypes resulted by *Riptortus linearis* infestation in both the NCT and FCT.

Correlation Study among Agronomic Characters with the Damage Intensity

The economic value of the soybean is in the quantity and quality of the soybean seeds. In the FCT, the number of punctures on the seeds was correlated with the intensity of seed damage (Table 2, above the diagonal), and the more seeds caused the higher the number of the punctures on the seeds. The relationship among characters in the NCT (Table 3, below the diagonal) was different from the pattern in the FCT, except the number of punctures on the seeds determines the seed damage intensity.

Interestingly, in the FCT, the intensity of seed damage was determined by the number of punctures on the seeds, and the number of punctures related to the number of seeds. Meanwhile, the pattern in the NCT, the intensity seed damage was also related to the number of punctures on the seeds, but the number of punctures was not related to the number of seeds. This reflects that there is no opportunity for host choice in the NCT, or other words, NTC provides heavier pressure and selection than the FCT. Thus, the NTC will produce genotypes with high resistance to pod-sucking pests [18].

TABLE 3. Correlation among agronomic characters with pod damage and seed damage in the FCT and NCT.

Character	PD	SD	PH	SW	PN	SN	NU
PD		-0.011	-0.058	-0.020	-0.025	-0.227	-0.189
SD	0.342		0.339	0.346	0.106	0.351	0.908**
PH	-0.295	-0.515		0.293	-0.253	0.260	0.432
SW	-0.571	-0.012	0.316		0.261	0.481	0.462
PN	-0.632*	-0.171	0.429	0.867**		0.305	0.147
SN	-0.422	0.019	0.288	0.959**	0.843**		0.640**
NU	0.171	0.907**	-0.477	0.284	0.058	0.3441	

FCT = above the diagonal, NCT = below the diagonal, PD = pod damage (%), SD = seed damage (%), PH = plant height (cm), SW = seed weight per plant (g), PN = pod number per plant, SN = seed number per plant, NU = number of punctures on the seeds. **Significant at 1% probability level ($p<0.01$). *Significant at 5% probability level ($p<0.05$).

Obtaining a soybean genotype that is resistant to pod-sucking pests is important because, in addition to maintaining yield potential, it has the potential to prevent negative environmental impacts and the occurrence of insecticide resistance. A survey of the use of several insecticides revealed that the insecticides beta-cyfluthrin and imidacloprid were less effective at suppressing the brown stink bug *Euschistus heros* infestation; it was suspected that the pests had undergone an immune process, or have the insecticide resistance [19]. Furthermore, it is possible to have a degree of dominance over a pod-sucking pest, and it has been reported in Argentina that *N. viridula* is currently considered to be of lower economic value [20]. On the other hand, there is a significant opportunity to increase soybean resistance to pod-sucking pests. As stated by Asadi *et al*. [16] that soybean resistance to pod-sucking bug *R. linearis* was controlled by two recessive genes located at different loci, and interacted with each other with epistatic dominant reaction. With an assumption that *B* and *C* genes were dominant, hence the resistance genes found in both soybean genotypes were *bbcc*. In this study, soybean genotypes resistant to pod-sucking pests are those that are consistently resistant to both FCT and NCT, as well as those that are resistant based on the intensity of the pod and seed damages.

CONCLUSION

A soybean genotype (G4) was identified as resistant to pod-sucking bug *R. linearis* based on the pod and seed damages caused by the insect pest both in the FCT and NCT methods, respectively. This resistant soybean genotype is potentially used as a resistance gene source for developing soybean lines resistance to pod-sucking insect pest. The intensity of pod damage caused by the pod-sucking bug *R. linearis* was higher than the intensity of seed damage caused by the insect. The damage intensity in the NCT method was higher than that obtained in the FCT method. The NCT method, therefore, should be more applicable to screen soybean genotypes for resistance against pod-sucking bug to obtain soybean genotypes with higher resistance levels.

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