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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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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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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FreeJanuary 2022

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

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

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

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

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-
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Sucrose and putrescine increased callus induction in tomato anther culture

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

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

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

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

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

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

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

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-
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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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FreeJanuary 2022

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

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

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-
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FreeJanuary 2022

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

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

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

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Ragapadmi Purnamaningsih and Suci Rahayu

Response of Gamma Ray Irradiation Derived-Cultures of Three Sugarcane Varieties to Drought Stress Induced by Polyethylene Glycol

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Abstract. Drought is one of limiting factor in sugarcane growth and productivity, therefore sugarcane which has high production in the dry season is needed. Somaclonal variation combined with *in vitro* selection could increase genetic variation, while those new variants can be selected *in vitro*. The purpose of this study was to evaluate the response of somaclones derived from gamma ray irradiation combined with *in vitro* selection by using polyethylene glycol (PEG). Three new varieties of sugarcane (AAS Agribun, CMG Agribun, and AMS Agribun) were used as plant materials. Embryogenic calli were induced using 3 mg/l 2,4-D and 3g/l. casein hydrolysate followed by gamma ray irradiation at doses of 10, 20, 30, 40, 50, and 60 Gy. After recovery step, calli were selected by using PEG 6000 10 and 15%. The results showed that AMS Agribun derived-cultures were growth faster to form callus, but they were most sensitive after treated with gamma ray irradiation. The highest percentage of callus regeneration were obtained from AAS Agribun mutants after selected with PEG. The highest number of mutant shoots tolerant to PEG were obtained from AAS Agribun and the lowest from CMG Agribun. The higher gamma ray irradiation dose more than 30 Gy and *in vitro* selection more than 10% inhibited the growth of cultures.

INTRODUCTION

Drought is one of factors that affect the rate of plant growth. The increase in earth temperature and changes in rain patterns cause the availability of groundwater to decrease, even causing drought in various regions, causing considerable losses. The availability of sufficient water is necessary for plant metabolism and physiological processes.

Many superior varieties of sugarcane have been developed, however, the yield obtained is still below its potential yield. One of the contributing factors is changes in agroclimatic conditions and weather anomalies. Increasing sugarcane production can be achieved if the factors supporting the growth of sugarcane such as the availability of water is available. Sugarcane requires sufficient water, where in the early stages of growth to lengthening of sugarcane stalks, water demand is generally relatively high and decreases when it enters the ripening phase. The rapid growth or extension phase is the most critical phase, with water requirements reaching up to 7.5–8.5 mm/day [1].

Sugarcane is a C4 plant group which is known to have an efficient photosynthetic system, but the rate of photosynthesis in sugarcane has decreased due to drought stress [2]. Water deficit will cause stomata closure which will reduce cellular CO2 concentration, while dehydration in leaf mesophyll cells can cause photosynthetic organs damage [3]. Plants will adapt to drought stress by accumulating water to delay or escape from stress [3], or by closing stomata mediated by ABA synthesis. This adaptation has implications for the accumulation of CO_2 gas which will reduce the rate of photosynthesis so that the growth rate decreases [4]. Some of the important agronomic characters that will be affected by drought stress include a decrease in internode diameter, stem height, and a decrease in root length [5].

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Conventional improvement of genetic traits of sugarcane is difficult because flowering induction must be done using specific techniques. Somaclonal variation, mutation, and *in vitro* selection techniques can be used to increase the genetic diversity of plants, especially plants that are propagated vegetatively. *In vitro* culture techniques provides new opportunities to improve plant genetics, especially for characters that are not available in germplasm. Somaclonal variation is an *in vitro* culture method that can be used to obtain new characters that are not available in existing germplasm sources [6]. The application of somaclonal variation techniques is often combined with artificial mutations to increase the chances of obtaining the desired lines. Induce mutation can be done by applying gamma ray irradiation to the cell population (callus). This technique can accelerate the acquisition of new lines with various characteristics or characteristics desired.

The selection method developed for drought resistance is selection using polyethylene glycol (PEG) to stimulate drought stress conditions. PEG has an ability to control the water potential reduction homogeneously linear to soil water potential [3]. The PEG concentrations of 5, 10 or 15% can effectively differentiate the drought tolerance of soybean genotypes similar with the field experiment result [7].

New varieties of sugarcane, namely AAS Agribun, CMG Agribun, and AMS Agribun, have a yield potential of more than 10% and productivity of more than 100 t/ha, higher than other varieties, therefore adding drought tolerance is very important so that potential productivity and yield can be achieved.

MATERIALS AND METHODS

Callus Induction

The plant material used was embryogenic callus induced from three new sugarcane varieties (AAS Agribun, CMG Agribun, and AMS Agribun). The plant material used was young, rolled leaves which were taken from the innermost part of the cane shoots. Prior to planting, the explants were washed with soap, then sterilized by soaking the explants in Benlate solution for 1 h, then followed by 70% alcohol, 30% chlorox, and 20% chlorox. The sterilized explants were then cut into pieces 0.5 cm in size and planted on the medium for callus induction.

The media used for callus induction was the basic medium of Murashige-Skoog (MS) with the addition of 3 mg/l 2,4-D and 3 g/l casein hydrolysate. In one bottle, four explants were planted. The bottle containing the explants were then placed on the culture rack in the culture room in the dark for optimal callus formation. The resulting callus was sub-cultured every 3 weeks using the same media to induce callus proliferation to produce embryogenic callus. Observations were made on callus formation time, callus color, and callus structure. A completely randomized design was used. Data were analyzed using ANOVA at the 95% confidence level. If there is a significant difference between treatments, then a further test is carried out using the Duncan's Multiple Range Test (DMRT) at the 95% confidence level.

Induction of Mutations in Callus Using Gamma Ray Irradiation

The callus formed was treated with gamma ray irradiation at doses (10, 20, 30, 40, 50, and 60 Gy), then the callus was transferred to MS medium without growth regulators. The number of replication for each dose was 15 calluses. This method was carried out on the callus of the three varieties used. The bottle filled with callus was placed in a culture rack using a TL lamp with an irradiation intensity of 1,000 lux for 16 h a day. Observations were made on the color of the callus and the appearance of green spots on the callus resulting from the gamma ray irradiation treatment. Color changes in callus were scored with a value of 1–5 (1 = shiny; 2 = yellowish; 3 = brownish; 4 = brown; 5 = black), while the presence or absence of a green spot on the callus was given a score of 1–2 (1 = there is a green spot; 2 = no green spot).

In Vitro Selection for Drought Tolerance

The mutated callus population was transferred to MS medium added with PEG 6000 (10 and 15%). The number of samples in each treatment was 10 clump callus with two replications. Selection on media containing PEG was carried out for 2 months. The bottle that has been planted with callus was placed in a culture rack using a TL lamp with an irradiation intensity of 1,000 lux for 16 h a day. Observations were made on the percentage of survival callus after treated with 10 or 15% PEG.

Putative Callus Regeneration of Somaclone Mutants and Root Induction

The media formulation used for callus regeneration was MS + 0.1 mg/l BA + 0.01 mg/l NAA. Shoots with good morphology were transferred into the medium to induce root formation using MS medium containing 1 mg/l NAA. The bottle containing the shoots was placed in a culture rack using a TL lamp with an irradiation intensity of 1,000 lux for 16 h a day. Observations were made based on the percentage of regenerated callus, number of shoots, number of roots, and root length. A completely randomized design was used with three factors. The first factor was the variety used (AAS Agribun, CMG Agribun, and AMS Agribun), the second factor was the dose of gamma ray irradiation (10, 20, 30, 40, 50, and 60 Gy), and the third factor was the concentration of PEG (10 and 15%). Data were analyzed using ANOVA at the 95% confidence level. If there is a significant difference between treatments, then a further test is carried out using the DMRT at the 95% confidence level.

RESULTS AND DISCUSSIONS

Callus is induced from the young leaves using a certain media composition. The leaves of the three varieties used develop callus with different initiation times, ranging from 2–3 weeks. The fastest callus initiation was obtained from AMS Agribun (13.4 days), which was significantly different from other varieties, followed by AAS Agribun, and the slowest was CMG Agribun (Table 1 and Fig. 1).

TABLE 1. Time of callus formation, callus color, and callus structure.

Variety	Time of callus formation (days)	Callus color	Callus structure
CMG Agribun	18.2 a	Brownish white	Friable
AAS Agribun	16.0 b	Brownish white	Friable
AMS Agribun	13.4 c	Brownish white	Friable

Means within a column having the same letter are not statistically significant (p = 0,05) according to Duncan's Multiple Range Test.

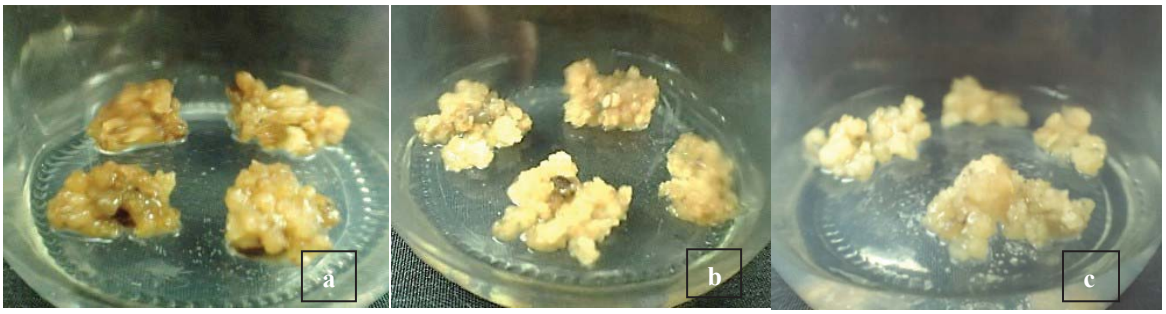

FIGURE 1. Induction of sugarcane embryogenic callus using MS containing 2,4-D. (a) AAS Agribun. (b) CMG Agribun. (c) AMS Agribun.

Gamma ray irradiation caused a change in callus color which varied depending upon the variety and the dose of gamma ray irradiation given. Gamma ray irradiation can cause cell damage so that cells released phenolic compounds as a self-defense mechanism. The more compounds released, the more callus color turn brown/black. The ability of cells to survive to this condition determines the regeneration ability of the callus which is indicated by the appearance of green spots on the callus (Fig. 2). The result shows that the AAS Agribun has the smallest color change score compared to the CMG Agribun and AMS Agribun. AMS Agribun were most sensitive to gamma ray irradiation treatment as indicated by the greatest callus color change score and the smallest green spots score.

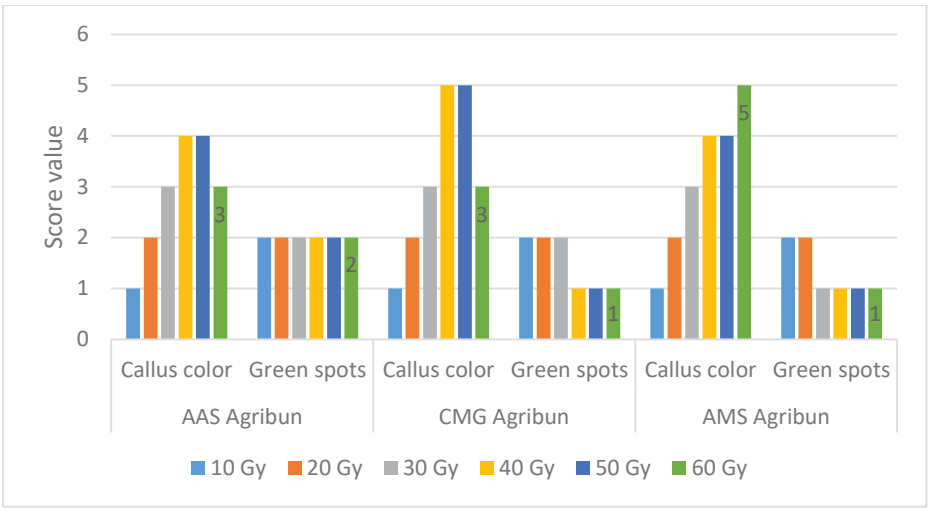

FIGURE 2. Scoring of color change in callus and appearance of green spots on callus after gamma ray irradiation treatment at a dose of 10 to 60 Gy.

TABLE 2. Percentage of survival callus after gamma ray irradiation and *in vitro* selection, 2 months after treatment.

Somaclonal mutants	Irradiation dosages (Gy)	Percentage of survival calli		
		0% PEG	10% PEG	15% PEG
AAS Agribun	0	90	0	0
	10	85	40	0
	20	80	50	50
	30	50	15	25
	40	30	15	10
	50	20	5	0
	60	20	12.5	0
CMG Agribun	0	85	0	0
	10	70	30	0
	20	60	30	0
	30	40	0	0
	40	25	0	0
	50	0	0	0
	60	0	25	0
AMS Agribun	0	90	0	0
	10	80	40	15
	20	100	15	12.5
	30	70	12.5	10
	40	40	0	0
	50	30	0	0
	60	10	0	0

Gamma ray irradiation dose affects the callus ability to survive. Increasing gamma ray irradiation dose could increase cell damage and decreasing the callus survival. The addition of PEG to the media further decreased the viability of the callus. PEG application on callus that were not treated with gamma ray irradiation caused 100% of callus death. Callus survival in media containing PEG 10% were higher compared to 15% (Table 2).

Regeneration ability of the mutants from CMG Agribun was the lowest compared to the other two varieties (Table 3). The gamma ray irradiation treatment seemed to further reduce its regeneration ability. The calli treated with gamma ray irradiation at a dose of 50 Gy were all unable to regenerate to form shoots. According to Daszkowska-Golec and Szarejko [3], response of callus to gamma ray irradiation depends on the explant source and

irradiation dose rate. The level of sensitivity of plants to gamma ray irradiation is influenced by the type of plant, the growth phase, the size and physiological conditions of the explants, which was varies between plant types and genotypes. High doses of irradiation can result in tissue death [8]. In mangosteen, increasing the irradiation dose decreases the ability of nodular callus to form shoots [9]. The application of gamma ray irradiation at a dose of 25 Gy reduced the shoot formation compared to the control.

TABLE 3. Regeneration of gamma ray-irradiated mutant callus in selection media with PEG.

Somaclonal mutants	Irradiation dosages (Gy)	PEG concentration (%)	Regeneration (%)	Shoots number
AAS Agribun	10	10	40	16
	10	15	30	16
	20	10	40	17
	20	15	30	15
	30	10	35	10
	30	15	25	6
	40	10	30	8
	40	15	25	6
	50	10	0	0
	50	15	0	0
	60	10	12.5	5
	60	15	0	0
CMG Agribun	10	10	30	10
	10	15	0	0
	20	10	35	12
	20	15	0	0
	30	10	0	0
	30	15	0	0
	40	10	0	0
	40	15	0	0
	50	10	0	0
	50	15	0	0
	60	10	25	5
	60	15	0	0
AMS Agribun	10	10	30	10
	10	15	35	10
	20	10	40	12
	20	15	12.5	5
	30	10	35	10
	30	15	0	0
	40	10	0	0
	40	15	0	0
	50	10	0	0
	50	15	0	0
	60	10	0	0
	60	15	0	0

Mutated callus that were still viable on the selection media using PEG (10 and 15%), not all of them could regenerate to form shoots. Callus regeneration ranges from 0 to 40%. The highest regeneration ability was conducted from AAS Agribun, while CMG Agribun had the lowest regeneration ability, besides that, the presence of PEG of 15% in the medium seemed to cause a high enough stress, thus reducing the cells ability to regenerate quite significantly. Table 3 shows that most of the mutated callus cannot regenerate to form shoots, this indicates that the calli have lost their ability to grow and develop into complete plants because the gamma ray irradiation given causes damage the shoot meristem of cells and plants lose the ability to regenerate. In addition, the presence of drought

stress in the growing media due to the use of PEG causes cells that do not tolerate to these conditions to be unable to form shoots (Fig. 3).

PEG with molecular weight of 6,000 is a natural polymer that is water-soluble and nonionic. PEG 6000 is found to mimic drought stress and results in lowering of plant's water potential due to osmotic stress [10, 11]. The highest number of shoots were obtained from AAS Agribun with 99 shoots, followed by AMS Agribun with 47 shoots and CMG Agribun with 27 shoots.

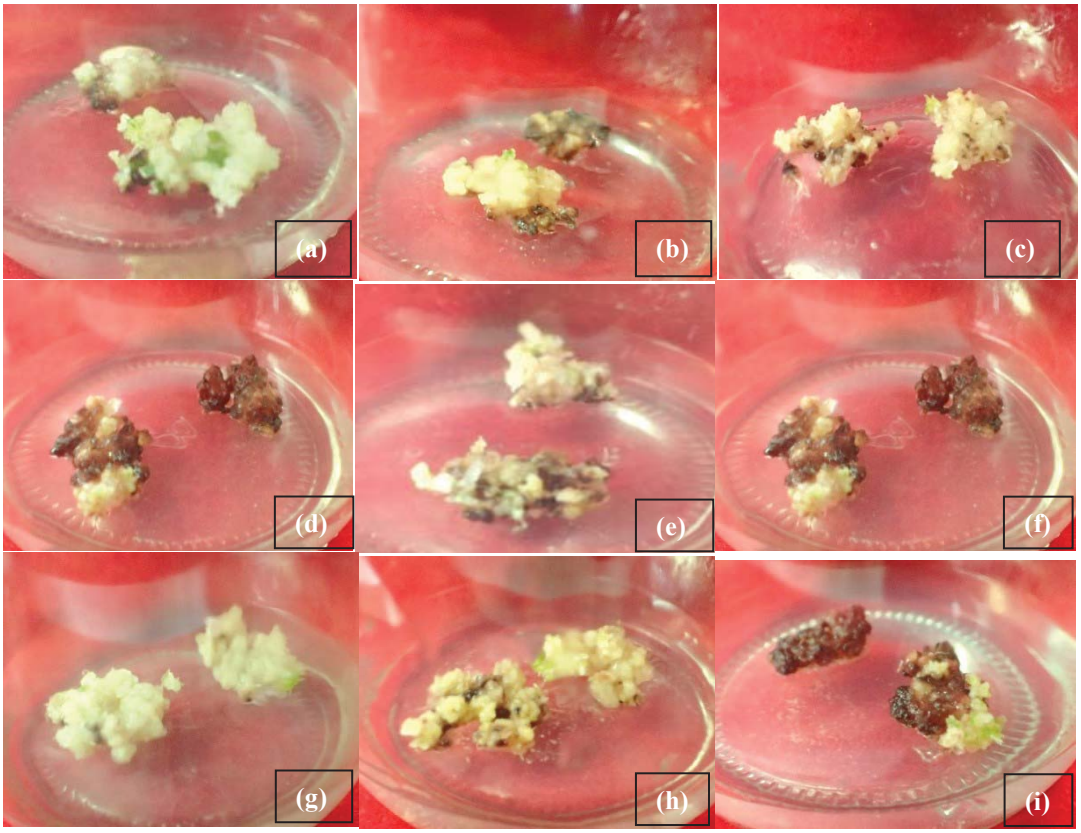

FIGURE 3. Regeneration of mutant calli of sugarcane after gamma ray irradiation and *in vitro* selection with 10% PEG. (a) AAS Agribun, 10 Gy. (b) AAS Agribun, 20 Gy. (c) AAS Agribun, 30 Gy. (d) CMG Agribun, 10 Gy. (e) CMG Agribun, 20 Gy. (f) CMG Agribun, 30 Gy. (g) AMS Agribun, 10 Gy. (h) AMS Agribun, 20 Gy. (i) AMS Agribun, 30 Gy.

Growth of shoot and root growth is presented in Table 4. There is a different growth response of the mutants. The highest shoots were produced from CMG Agribun at dose of 10 Gy with PEG concentration of 10%, while the shortest shoots were obtained from AAS Agribun of 30 Gy and selected with 10% PEG. The highest number of roots was obtained from AAS Agribun of 10 Gy and 10% PEG, which were not significantly different with AMS Agribun of 10 Gy and 10% PEG and CMG Agribun of 10 Gy and 10% PEG. The longest roots were obtained from AMS Agribun of 10 Gy dose of gamma ray irradiation and selected with 10% PEG (3.84 cm), while the shortest roots were obtained from AAS Agribun of 40 and 60 Gy with 10 and 15% PEG. Gamma ray irradiation causes genetic changes in the cells and increasing genetic tolerance of the cells to drought stress [12, 13]. The higher the gamma ray irradiation dose given, the lower the growth rate of shoots and roots. High doses of gamma ray irradiation will generally cause cells damage and made cells lose the ability to growth. The application of gamma ray irradiation at doses of more than 30 Gy caused significant inhibition of shoot and root growth.

TABLE 4. Growth of shoots and roots of mutant sugarcane irradiated by gamma rays and selected with PEG.

Somaclonal mutants	Irradiation dosages (Gy)	PEG concentration (%)	Shoot height (cm)	Roots number	Root lenght (cm)
AAS Agribun	10	10	3.00 b	8.00 a	3.48 b
	10	15	2.95 b	6.00 bc	2.40 d
	20	10	2.56 cde	6.00 bc	2.00 ef
	20	15	2.00 gh	5.74 bc	2.50 d
	30	10	1.80 h	5.00 bc	2.00 ef
	30	15	2.02 gh	5.74 bc	1.92 f
	40	10	2.20 fg	4.00 d	1.50 g
	40	15	2.00 gh	4.74 cd	1.76 fg
	60	10	2.30 ef	4.00 d	1.46 g
CMG Agribun	10	10	3.44 a	7.00 ab	3.46 b
	20	10	2.6 cd	5.74 bc	3.42 b
	60	10	2.00 gh	5.00 cd	2.00 ef
AMS Agribun	10	10	3.00 b	8.00 a	3.84 a
	10	15	2.80 bc	6.00 bc	3.00 c
	20	10	2.36 def	5.74 bc	2.94 c
	20	15	2.12 fg	5.00 cd	2.98 c
	30	10	2.50 de	5.74 bc	2.30 de

Means within a column having the same letter are not statistically significant (p = 0,05) according to Duncan's Multiple Range Test.

Drought stress causes inhibition of cell division, enlargement, and elongation due to the low water content available, thereby reducing cell turgor [14]. The decrease in turgor pressure in plant cells also causes inhibition of the process of cell division and enlargement [3], so that the plant becomes short. Drought stress can also inhibit the process of cell division and enlargement in the roots, as a result of which root growth is inhibited. Roots are an important organ in plants, especially for absorbing water and nutrients in the planting medium. More plants develop root systems in response to nutrient deficiencies and drought [15].

CONCLUSION

AMS Agribun derived cultures were faster to form callus compared to AAS Agribun and CMG Agribun, but the callus were most sensitive after treated with gamma ray irradiation. The highest percentage of callus regeneration were obtained from AAS Agribun mutants after selected with PEG. The highest number of mutant shoots tolerant to PEG were obtained from AAS Agribun and the lowest from CMG Agribun. The higher gamma ray irradiation dose more than 30 Gy and *in vitro* selection more than 10% inhibited the growth of cultures.

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