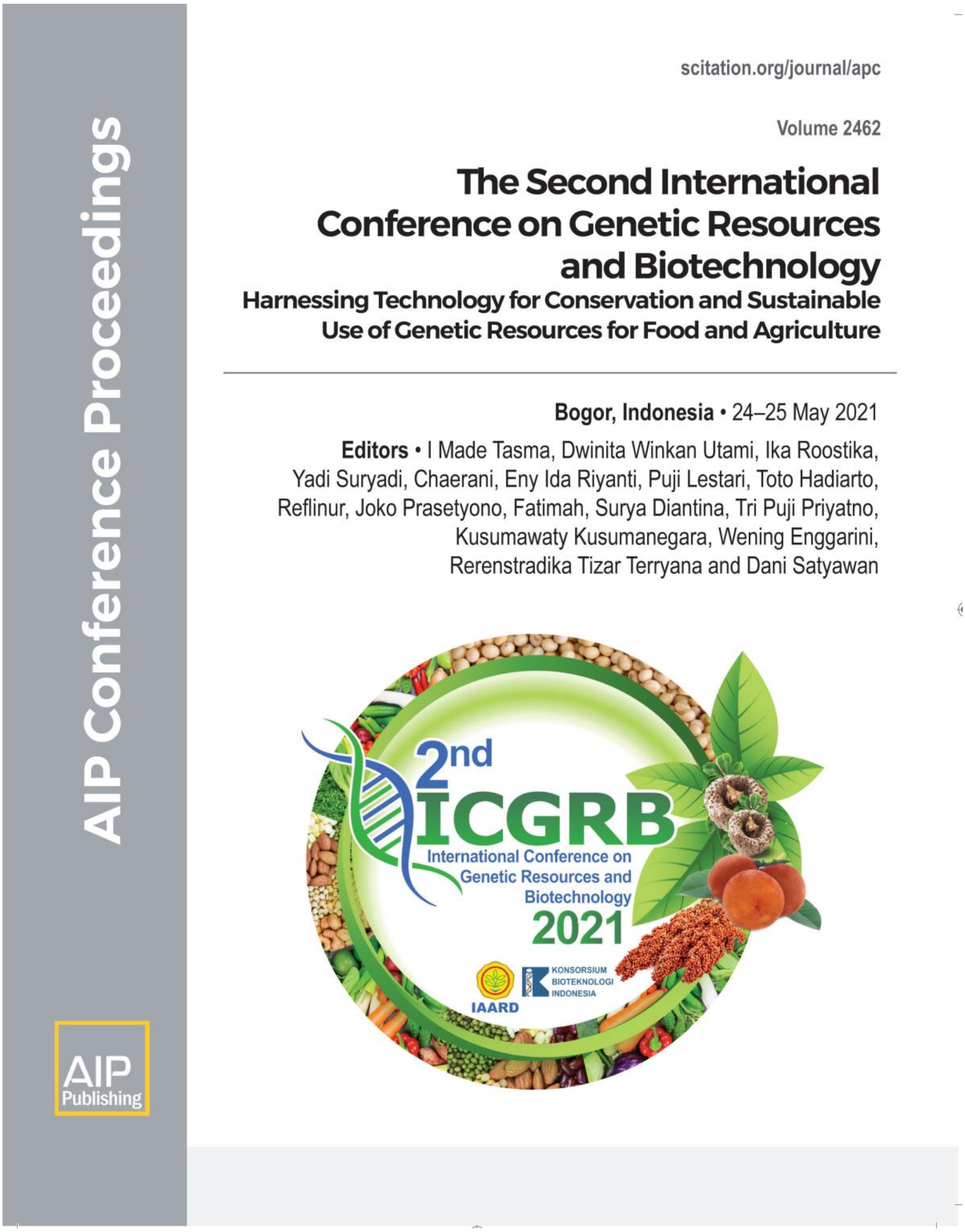

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Volume 2462

The Second International Conference on Genetic Resources and Biotechnology

Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Bogor, Indonesia • 24–25 May 2021

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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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Harnessing plant genetic resources through biotechnology for food security in Indonesia

Mastur, Reflinur, Nurul Hidayatun, Sustiprijatno, Fatimah, Tri Puji Priyatno and Puji Lestari
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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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-
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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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-
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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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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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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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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

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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

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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

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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
  - E-READER
  - ADD TO FAVORITES
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  - EXPORT CITATION

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

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

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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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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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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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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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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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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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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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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
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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
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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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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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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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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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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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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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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
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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

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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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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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The growth of garlic internal sprout on different storage condition

Chotimatul Azmi, Imas Rita Saadah, Nazly Aswani and Asih Kartasih Karjadi
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Muhamad Sabran, Dwinita Wikan Utami and Susilawati
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Edy Listanto, Ahmad Warsun, Ahmad Dadang, Eny Ida Riyanti, Saptowo Jumali Pardal, Sustiprijatno and Mastur
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Molecular diversity comparison in local rice accessions originated from Kalimantan and other islands of Indonesia

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

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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
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Ida Rosdianti, Dani Satyawan, Muhamad Yunus and Dwinita Wikan Utami
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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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-
    - PDF
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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
AIP Conference Proceedings **2462**, 030007 (2022); https://doi.org/10.1063/5.0075160

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-
  - PDF
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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
AIP Conference Proceedings **2462**, 030008 (2022); https://doi.org/10.1063/5.0075392

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-
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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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-
  - PDF
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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

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-
  - PDF
  - E-READER
  - ADD TO FAVORITES
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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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I Made Tasma, Dwinita Winkan Utami, Ika Roostika, Yadi Suryadi, Chaerani, Eny Ida Riyanti, Puji Lestari, Toto Hadiarto, Reflinur, Joko Prasetyono, Fatimah, Surya Diantina, Tru Puji Priyanto, Kusumawaty Kusumanegara, Wening Enggarini, Rerenstradika Tizar Terryana and Dani Satyawan

Volume number: 2462
Published: Jan 19, 2022

DISPLAY :

- 20
- 50
- 100
- all

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

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

Sri Koerniati

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

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

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

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

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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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-
  - PDF
  - E-READER
  - ADD TO FAVORITES

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- EXPORT CITATION

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

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

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

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

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

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-
    - PDF

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

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

FreeJanuary 2022

Sucrose and putrescine increased callus induction in tomato anther culture

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

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

FreeJanuary 2022

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

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

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

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

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

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

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

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

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

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

FreeJanuary 2022

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

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

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-
    - PDF
    - E-READER
    - ADD TO FAVORITES
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Biofertilizer increases nutrient use efficiency (NUE) of nitrogen, phosphorus, and potassium at leaves level of *Artemisia annua* L.

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

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

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

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

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-
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  - ADD TO FAVORITES
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FreeJanuary 2022

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

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

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

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

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-
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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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-
  - PDF
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  - ADD TO FAVORITES
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Physicochemical characteristics of yoghurt from various beans and cereals

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

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

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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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-
  - PDF
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  - ADD TO FAVORITES
  - SHARE
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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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-
  - PDF
  - E-READER
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Genetic Variation of Adan, a Krayan Local Rice Mutant, Using Microsatellite Markers

Joko Prasetiyono[1, a)], Tio Fadel Rafsanjani[2], Tri Aminingsih[2], Tasliah[1]
and Sugiono Moeljopawiro[1]

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Abstract. One of the famous local rice in the border region of North Kalimantan Province with Malaysia is Adan rice. However, the long growth duration of Adan rice (around six months) means that local farmers can only plant this rice once a year. Efforts to shorten the life of Adan rice have been carried out using gamma-ray radiation, and resulted an early heading mutant lines with four-month old possessing physical characteristics similar to Adan rice. This study aimed to evaluate genetic variaton on selected Adan rice mutants using SSR markers. This research was conducted at the Molecular Biology Laboratory, Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, from February to July 2015. A total of eight selected short-duration mutants and Adan cultivar (its wild type) were genotyped using seventy one SSR markers located on all rice chromosomes. Genetic similarity among tested samples was determined using the Dice coefficient, followed by grouping the samples using the UPGMA method facilitated in NTSYSpc-2.02 software. The seventy one SSR markers produced 1–9 bands, 1–10 alleles with the PIC value ranged from 0.00–0.97. The dendrogram grouped the mutant lines into two groups at a similarity level of 65%. The first group contained Adan rice, A28, A25, and A27 rice mutant lines, while the second ones consisted of A51, A88, A93.1, A93.2, and A50 rice mutant lines. The group I can be used as candidates for mutant development at Krayan District, where Adan rice is usually planted. This research also showed that SSR markers can be used to identify the genomic changes in rice mutants.

INTRODUCTION

Adan rice is a local rice cultivar which only recently be grown in Krayan Subdistrict, Nunukan Regency, North Kalimantan Province (formerly part of East Kalimantan Province). Adan rice has a distinctive taste that will change when it grown outside the region. Adan rice is usually grown organically, and only planted once in a year due to its long life (around 5−6 months). Adan rice is also the main consumption for the officials and people of Brunei Darussalam. Even more, the Sultan of Brunei has often specially ordered certain rice for consumption of the royal family. In contrast, Krayan residents rarely consume Adan rice as their daily menu [1].

Efforts to shorten the life of Adan rice have been initiated by the East Kalimantan Provincial Government since 2011 [2] with the hope that Adan rice can be planted twice in a year order to increase its production. Prasetiyono *et al*. [3] reported that shortening the lifespan of Adan rice has been done through mutation breeding using rice seeds irradiated with gamma rays doses of 0, 50, 100, 150, 200, and 250 Gy and resulted line an early maturity than the parent.

Adan rice mutants need to be further investigated using molecular markers to elucidate the change in the selected rice mutant lines by gamma-rays. Simple sequence repeats (SSR) or microsatellites markers is one of the effective markers in detecting the result of mutations. Variations in microsatellites are considered to be replication errors, rearrangements, due to insertions or deletions, or substitution mutations. SSR markers are located in all genome area, even the coding and non-coding, so they are not influenced by the environment [4]. The SSR markers have

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been widely used to identify mutants, for example, Singh *et al*. [5] has successfully identified soybean mutants, and Yasmeen *et al*. [6] applied this marker to identify sugarcane mutants. Application of SSR markers in differentiate mutations in rice were also widely reported [6, 7, 8, 9]. This study aimed to evaluate genetic variaton on selected Adan rice mutants using SSR markers.

MATERIALS AND METHODS

This research was conducted at the Molecular Biology Laboratory, Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, from February to July 2015. The genetic materials used in this study consisted of eight selected rice mutant lines of Adan rice and the parent (Table 1). These mutants were selected mutants from the first generation (M_1), where Adan rice seeds were irradiated at the Center for Isotope and Radiation Application, National Atomic Power Agency, Jakarta. Selection of rice mutant lines on the basis of plant age and morphological traits at the M_1 to M_3 generation was carried out in Bogor, while that at the M_4 to M_5 was in Krayan. The eight M_5 rice mutant lines used in present study showed early maturity and they had very similar performance with as parent or wild type. The molecular markers used to identify mutants were 71 SSR markers that spread across the 12 rice chromosomes (Table 2).

TABLE 1. List of plant genetic materials used in present study.

No.	Lines	Generation	Dose
1	Adan	Parent	0 Gy
2	A25	M_5	100 Gy
3	A27	M_5	100 Gy
4	A28	M_5	100 Gy
5	A50	M_5	150 Gy
6	A51	M_5	150 Gy
7	A88	M_5	150 Gy
8	A93.1	M_5	150 Gy
9	A93.2	M_5	150 Gy

Molecular Analysis

Two-week old leaves of each sample were used as the source for DNA isolation. The total DNA from these plants was isolated according to the modified Dellaporta method [10]. PCR was carried out in a 20 µl of total volume containing 1× buffer, 100 µM dNTPs, 0.5 µM primers (F and R), 50−100 ng DNA, and 1 unit of Taq DNA polymerase.

The PCR program applied was a temperature of 94°C for 5 minutes for initial denaturation, the followed by 35 cycles of 94°C for 60 seconds for denaturation, 55°C for 60 seconds for primer annealing, and 72°C for 2 minutes for primer extension. The final primer extension was carried out at 72°C for 7 minutes. The PCR results were separated using 8% polyacrylamide gel electrophoresis for 2 hours at a voltage of 80 volts. DNA staining was done by immersing the gel in ethidium bromide solution and documented using Bio-Rad Chemidoc Imaging System.

Data Analysis

Data analysis was performed by making a score from the resulting bands, a value of 1 for the presence of a band, and a value of 0 if there is no band observed at the same alleles. The scoring which included the number of bands, the number of alleles, and the Polymorphism Information Content (PIC) was calculated manually in the Microsoft Excel program. The PIC value was calculated based on the following formula as described by Hildebrand *et al*. [11]:

$$\text{PIC} = 1 - \sum_{i=1}^{n} p_i^2$$

where PIC = Polypmorphism Information Content, i = allele i in marker j, n = number of alleles in marker j, and p = allele frequency.

Information on genetic relationship among the mutants was obtained by analyzing binary data using the Dice coefficient, followed by grouping using the Sequential Agglomerative Hierarchical Non-overlapping (SAHN) technique and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Those programs are in NTedit and NTSYSpc-2.2 [12].

TABLE 2. List of SSR primers used in the study.

No.	Primer	cM	Chrom	No.	Primer	cM	Chrom
1	RM476A	16.3	1	37	OSR21	145.2	6
2	RM272	37.3	1	38	RM320	40.7	7
3	RM23	71.6	1	39	RM70	64.5	7
4	RM488	101.4	1	40	RM47	84.95	7
5	RM543	145.6	1	41	RM172	115.95	7
6	RM431	178.3	1	42	RM52	15.3	8
7	RM236	10.6	2	43	RM25	52.2	8
8	RM174	47.5	2	44	RM137	69	8
9	RM262	78.4	2	45	RM350	78.4	8
10	RM183	104.2	2	46	RM230	112.2	8
11	RM525	143.7	2	47	RM281	128.6	8
12	RM523	11	3	48	RM524	13.2	9
13	OSR13	53.1	3	49	RM321	32.1	9
14	RM232	78.7	3	50	RM409	45.6	9
15	RM125	89	3	51	RM434	57.7	9
16	RM16	131.5	3	52	RM205	114.7	9
17	RM347	144	3	53	RM222	11.3	10
18	RM448	181.4	3	54	RM311	25.2	10
19	RM565	215.5	3	55	RM467	46.8	10
20	RM551	14.9	4	56	RM184	58.3	10
21	RM185	50.8	4	57	RM294A	87.1	10
22	RM119	63.8	4	58	RM20B	0	11
23	RM252	99	4	59	RM332	27.9	11
24	RM317	118.3	4	60	RM120	41.7	11
25	RM131	148.8	4	61	RM479	50	11
26	RM413	26.7	5	62	RM229	77.8	11
27	RM437	43.4	5	63	RM187	89.7	11
28	RM430	76.7	5	64	RM254	110	11
29	RM440	92.7	5	65	RM415	0	12
30	RM178	118.8	5	66	RM558A	9.2	12
31	RM538	132.7	5	67	RM19	20	12
32	RM587	10.7	6	68	RM247	32.3	12
33	RM276	40.3	6	69	RM277	57.2	12
34	RM3	74.9	6	70	RM309	74.5	12
35	RM275	108.3	6	71	RM235	102.6	12
36	RM30	125.4	6				

cM = centimorgan, Chrom = chromosome.

RESULTS AND DISCUSSIONS

This research activity has succeeded in selecting early maturing Adan rice mutant lines. Based on the selection of mutant lines from 2011 to 2014, there were mutants that were able to survive until harvested at radiation doses of 100 and 150 Gy, while seeds of 200 and 250 Gy were not obtained. The mutants showed early maturation (Fig. 1), so this result gives hope for farmers in Krayan District to plant Adan rice twice a year.

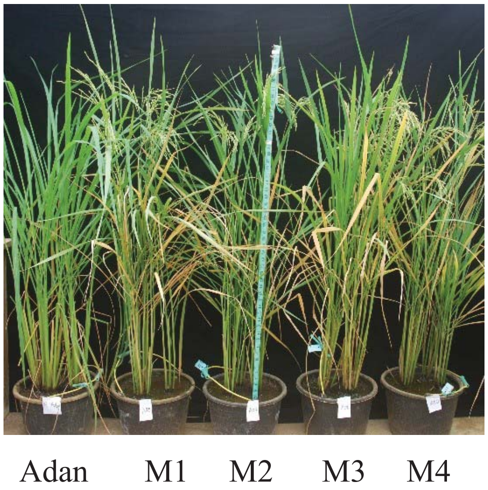

FIGURE 1. The performance of rice mutant lines with early flowering (M1-M4) in comparison to their parent, Adan rice.

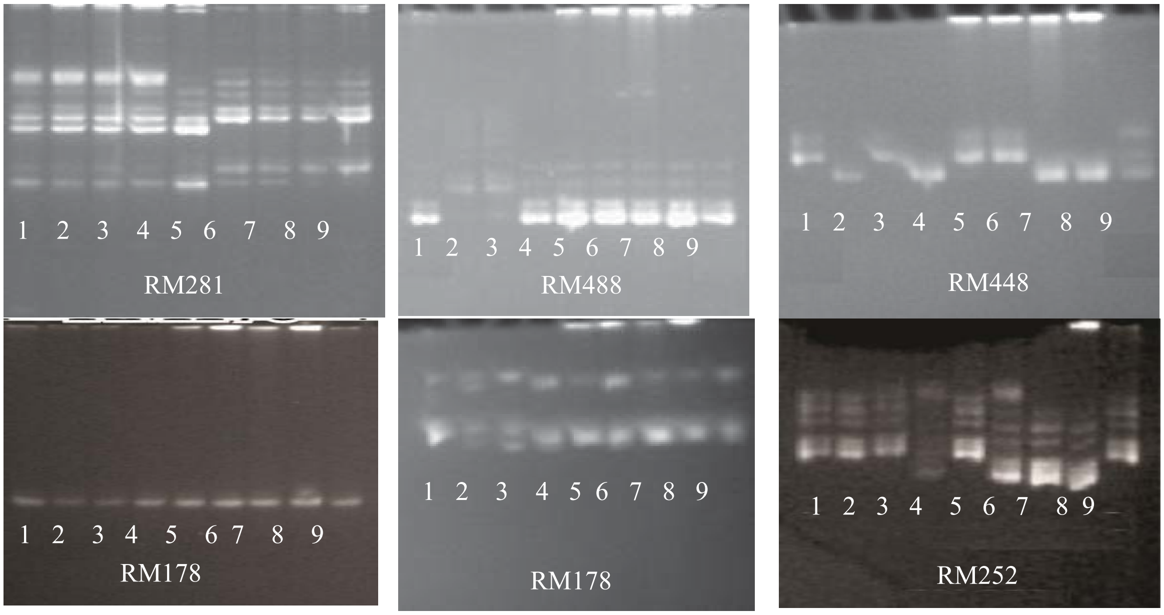

FIGURE 2. Examples of PCR performance of some SSR markers in Adan rice mutant lines. 1 = Adan, 2 = A25, 3 = A27, 4 = A28, 5 = A51, 6 = A88, 7 = A93.1, 8 = A93.2, 9 = A50.

The SSR markers used in this study resulted in alleles variation produced in eight selected mutant lines (Fig. 2). This mutation event caused either the addition or reduction of DNA bases in the Adan rice mutants, which indicated by different the position of the DNA bands that were not parallel to the Adan rice. In the RM281 marker, there was an addition of base (insertion) in the A88, A93.1, A93.2, and A50 mutant lines, while in the RM488 marker, there was also additional bases in the A25 and A27 mutant lines. Base reduction events (deletions) occurred in markers RM448 on A25, A28, A93.1, A93.2, and A50 mutant lines. Mutations did not occur in the RM178 and RM 437 markers. Both loci were on chromosome 5 (Table 1). Naem *et al*. [13] stated that gamma rays can randomly destroy nitrogen bases (A, G, T, and C), so that there will be base damage in the mutants randomly. This mutation treatment in rice can cause variations in the agronomic character. It is proven that early maturity mutants were produced.

TABLE 3. The number of bands, alleles, and PIC values of the resulting SSR bands.

No.	Primer	Bands number	Alleles number	PIC value	No.	Primer	Bands number	Alleles number	PIC value
1	RM476A	9	9	0.66	37	OSR21	4	4	0.35
2	RM272	1	1	0.56	38	RM320	2	5	0.85
3	RM23	3	3	0.70	39	RM70	2	2	0.85
4	RM488	4	7	0.58	40	RM47	1	6	0.54
5	RM543	4	4	0.55	41	RM172	2	2	0.45
6	RM431	4	4	0.24	42	RM52	2	2	0.97
7	RM236	3	7	0.91	43	RM25	1	3	0.72
8	RM174	3	3	0.66	44	RM137	4	7	0.49
9	RM262	3	3	0.65	45	RM350	2	3	0.59
10	RM183	4	7	0.83	46	RM230	2	4	0.89
11	RM525	2	3	0.63	47	RM281	5	10	0.44
12	RM523	1	1	0.56	48	RM524	2	2	0.77
13	OSR13	4	4	0.62	49	RM321	4	6	0.86
14	RM232	4	4	0.82	50	RM409	1	1	0.00
15	RM125	5	5	0.75	51	RM434	1	1	0.00
16	RM16	1	2	0.90	52	RM205	1	3	0.86
17	RM347	4	4	0.59	53	RM222	2	2	0.49
18	RM448	1	3	0.86	54	RM311	3	4	0.45
19	RM565	5	6	0.49	55	RM467	1	1	0.00
20	RM551	5	5	0.00	56	RM184	5	6	0.56
21	RM185	3	5	0.90	57	RM294A	3	4	0.90
22	RM119	1	1	0.00	58	RM20B	4	6	0.65
23	RM252	5	8	0.69	59	RM332	4	6	0.79
24	RM317	2	2	0.00	60	RM120	3	3	0.27
25	RM131	2	3	0.91	61	RM479	4	5	0.89
26	RM413	1	3	0.91	62	RM229	1	2	0.75
27	RM437	3	3	0.32	63	RM187	7	8	0.79
28	RM430	4	4	0.22	64	RM254	3	5	0.91
29	RM440	2	3	0.72	65	RM415	3	3	0.50
30	RM178	2	2	0.49	66	RM558A	5	5	0.41
31	RM538	1	3	0.86	67	RM19	4	5	0.73
32	RM587	6	6	0.65	68	RM247	2	3	0.91
33	RM276	1	1	0.89	69	RM277	4	4	0.42
34	RM3	3	4	0.70	70	RM309	3	3	0.07
35	RM275	3	6	0.76	71	RM235	2	2	0.54
36	RM30	4	4	0.72					
						Average	2.99	3.96	0.61
						Range	1–9	1–10	0.00–0.97

PIC = Polymorphism Information Content.

Based on the band pattern, 6 markers (8.45%) produced monomorphic bands with a total band of 1−5. This showed that there is no mutation event in the locus area. According to Table 3, there are variations for each marker, both in the number of bands, the number of alleles, or the PIC value. The number of bands and the number of alleles are not always the same, because the number of alleles shows the resulting banding pattern. The number of alleles will always be equal or greater to the number of bands. This variation of bands or alleles depends on the number of markers and the variation of genotypes tested. In this study, the genotypes used did not have many variations because the mutant lines were derived from the Adan rice parent. The average number of alleles produced was 3.958 per marker with the range of 1−10 alleles. These results are similar to those of Nugroho *et al*. [14] which used 24 rice genotypes and 15 markers with a higher allele mean of 3.9 per marker, with the range of 2−10 alleles. The use of labeled SSR markers will increase the variety of alleles obtained [15].

The PIC value in this study showed a wide value ranged from 0.00 to 0.97. Six markers have a PIC value of 0.00, indicating that they produced bands in all genotypes with the same position (monomorphic). The highest PIC value (0.97) was obtained at the RM52 marker with two bands and two alleles. The use of a more sensitive method (labeled SSR) can increase the minimum PIC value, for example, 0.021 in mango [15] and 0.33 in rice [16]. The term PIC was originally published by Botstein *et al*. [17] with a simplified formula by Hildebrand *et al*. [11]. This value is intended to inform the level of polymorphism of the markers used. Based on the criteria of Hilderabrand *et al*. [11] (PIC>0.7 = very informative, 0.7>PIC>0.44 = moderate informative, and PIC<0.44 = less informative), of the 71 SSR markers used in the study can be grouped into three categories, namely 14 markers as less informative, 25 as medium informative, and 32 as very informative (Table 3).

Based on the genetic relationship analysis (Fig. 3), a dendrogram was generated, dividing the rice samples into two groups at a similarity level of 65%, namely group I dan group II. The group I consisted of Adan rice and A28, A25, and A27 mutant lines, while the group II consisted of A51, A88, A93.1, A93.2, and A50 mutant lines. The similarity matrix (Table 4) showed that A27 and A28 mutant lines had the highest similarity level compared to Adan rice. This indicates that the genome of these two mutant lines are very similar to that of the Adan rice parent, even though short duration mutations have occurred. The A27 and A28 mutant lines can potentially be used as candidate lines for further development in Krayan District. In the study of the genetic relationship of rice (not mutation treatment), a study by Nugroho *et al*. [14] found that the percentage of similarity amounted to 63% with a more diverse sample composition. The study of Pathaichindachote *et al*. [18] which used *indica* and *japonica* grains from Thailand reported that the grouping of the two types of rice occurs at a similarity level of 11%.

TABLE 4. Similarity matrix between mutant lines and the parent.

	Adan	A25	A27	A28	A51	A88	A93.1	A93.2	A50
Adan	1.00								
A25	0.65	1.00							
A27	**0.71**	0.799	1.00						
A28	**0.71**	0.64	0.65	1.00					
A51	0.698	0.58	0.62	0.65	1.00				
A88	0.67	0.59	0.64	0.63	0.84	1.00			
A93.1	0.64	0.57	0.59	0.63	0.74	0.82	1.00		
A93.2	0.63	0.55	0.58	0.58	0.74	0.83	0.88	1.00	
A50	0.63	0.58	0.59	0.63	0.73	0.73	0.72	0.69	1.00

All mutant lines in group I come from 100 Gy gamma-ray doses, while the other mutant lines (group II) come from 150 Gy gamma-ray doses (Table 1). This showed that a lower dose of gamma rays has lower damaging effect on the genome. Sequencing of the mutant's entire genome has begun to improve accuracy of clustering [19, 20]. However, for the initial detection of mutants, the SSR markers can still be used.

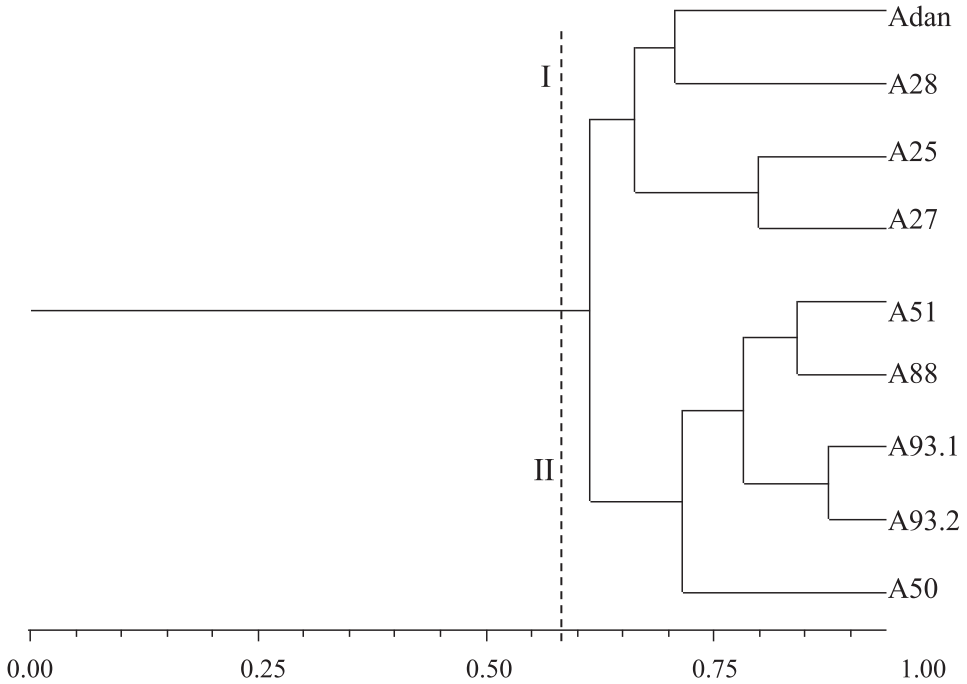

FIGURE 3. Dendrogram of Adan rice mutants using 71 SSR markers.

CONCLUSION

SSR markers are probably effective in differentiating Adan rice parents from mutants. The A27 and A28 mutant lines can be used as candidates for early maturity lines that can be developed in Krayan District.

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