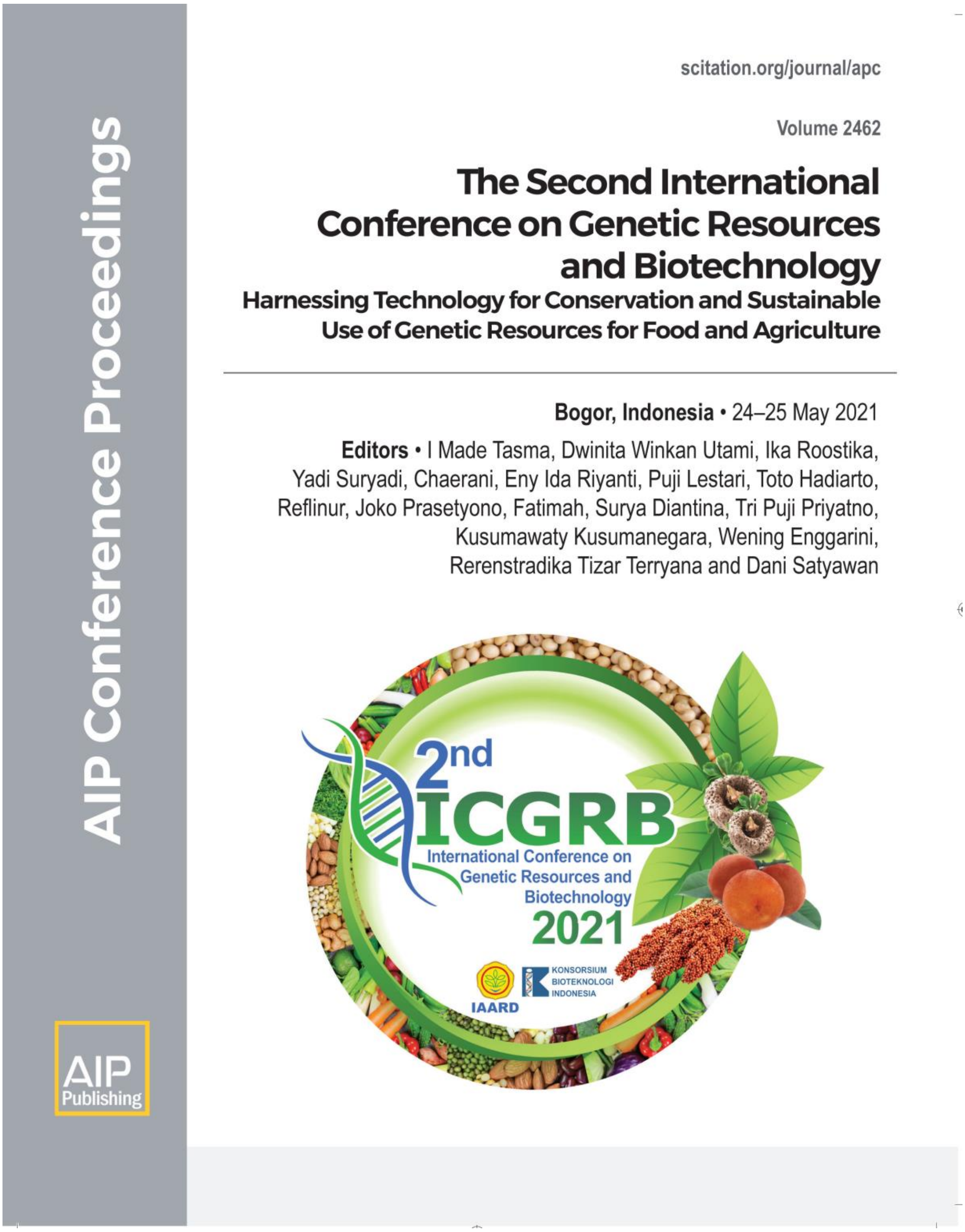

January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

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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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- 20
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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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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
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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
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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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-
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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
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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
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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
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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
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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
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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
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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
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-
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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
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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
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Evaluation of morpho-agronomical characters and grain quality of red rice lines

Heni Safitri and Puji Lestari
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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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  - ADD TO FAVORITES
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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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-
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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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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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Current status of tidal swamp rice varieties and its improvement for Fe toxicity tolerance and biofortification

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

Tasliah, Kurniawan Rudi Trijatmiko and Joko Prasetiyono
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Hybrid purity assessment in F_1 hybrids segregating for phytophthora root rot resistance genes of chili pepper (*Capsicum annuum* L.)

Fatimah, Reflinur, Joko Prasetiyono, Wartono, Kristianto Nugroho, Rinda Kirana, Dani Satyawan, Rerenstradika Tizar Terryana, Aqwin Polosoro, Puji Lestari and I. Made Tasma
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Characterization of genomic variation on three Indonesian oil palm genotypes analyzed using next-generation sequencing HiSeq

I. Made Tasma, Habib Rijzaani, Dani Satyawan, Ida Rosdianti, Edy Supriyanto and Razak Purba
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Cytological and molecular identifications of seedless tangerine derived from endosperm culture

Chaireni Martasari, Mia Kosmiatin, Ali Husni, Kurniawan Budiarto and Innez Candri Gilang Purnama
AIP Conference Proceedings **2462**, 030009 (2022); https://doi.org/10.1063/5.0076395

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Improvement of sex determination of salak plant using sequence characterized amplified regions

Reflinur, Ma’sumah, Namira Nur Arfa, Budi Setiadi Daryono and Azis Natawijaya
AIP Conference Proceedings **2462**, 030010 (2022); https://doi.org/10.1063/5.0075698

Table of Contents

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

_PREV

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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

FreeJanuary 2022

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

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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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FreeJanuary 2022

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
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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
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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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Morphological characters and efficacy of thirteen entomopathogenic fungi of *Aschersonia aleyrodis* Webber isolates on whitefly (*Bemisia tabaci* Gennadius)

Yusmani Prayogo, Marida Santi Yudha Ika Bayu, Sri Wahyuni Indiati and Made Jana Mejaya
AIP Conference Proceedings **2462**, 060008 (2022); https://doi.org/10.1063/5.0076067

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  - 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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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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Genetic relationship of pigmented rice (*Oryza sativa* L.) collected from Eastern Indonesia based on morpho-agronomical traits and SSR markers

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Yusi Nurmalita Andarini, Willy Bayuardi Suwarno, Hajrial Aswidinnoor, et al.

Genetic Relationship of Pigmented Rice (*Oryza sativa* L.) Collected from Eastern Indonesia Based on Morpho-Agronomical Traits and SSR Markers

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Abstract. Due to its nutritional content and beneficial health, black and red rice, which belong to pigmented rice, has become more popular. Research on pigmented rice in Indonesia has not been intensively carried out, so information on the genetic diversity of pigmented rice is very important for an effective breeding program. This research was conducted to analyze the genetic diversity of 15 accessions of local pigmented rice collected from Eastern Indonesia (Sulawesi and East Nusa Tenggara) in IAARD-ICABIOGRAD Gene Bank based on 33 morpho-agronomical characters and 24 SSR markers. The experiment was designed in a randomized complete block design with three replications. The morpho-agronomical observation was performed based on a standard descriptor for rice. The morpho-agronomic characters of all observed accessions varied, indicating high diversity. Based on the characteristic of SSR markers, the average allele number, major allele frequency, gene diversity, heterozygosity, and the PIC were 4.88, 0.47, 0.66, 0.02, and 0.62, respectively. The similarity coefficient between accessions based on morpho-agronomic characters and SSR markers was still quite high (80%). PCoA and phylogenetic analyses based on the results of combined SSR and morpho-agronomic data formed three different groups at a cophenetic distance of 0.22. The result of this study is expected to provide a better understanding of the genetic characteristics of the accessions for further use to support the PGR breeding program.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple crops for most people in the world [1]. As the main cereal crop, rice can adapt widely to geography, ecological, and climatic conditions [2]. Based on the color of the epidermis or aleuron, rice germplasm can be grouped into white, brown, red, and dark purple or black epidermis groups depending on the color pigments, especially anthocyanins in the pericarp layer [3]. Most people in developing countries, especially in Indonesia, chose white rice as the main staple food. In line with the increasing public preference for a healthy lifestyle, currently pigmented rice is becoming popular and widely consumed by the public as a functional food because it is beneficial for health. Pigmented rice contains anthocyanins that can function as antioxidants, which are components of bioactive nutrients that are beneficial to health [4].

Plant breeding activities in the context of genetic improvement require a wide genetic diversity as the basis for determining crossbred parents to develop desired traits such as rice color, productivity, rice quality, and resistance to pests and rice diseases [5]. The diversity of pigmented rice at the global level, especially in Asia, is known to be quite high [6]. The local pigmented rice varieties originating from Indonesia have a very diverse diversity which can be a valuable source for crop improvement. In their natural habitat, Indonesian local pigmented rice is increasingly

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rare because almost all farmers grow new varieties of rice, including hybrid rice, only a small proportion of them cultivate local varieties of pigmented rice. The use of local varieties in plant breeding programs is widely recommended to broaden the genetic background of superior varieties to be produced [7]. However, currently, research related to local pigmented rice in Indonesia has not been widely carried out, while the use of local pigmented rice germplasm will be possible if its characteristics are known.

Analysis of genetic diversity of existing germplasm can be carried out morpho-agronomically, isozyme, or with molecular markers to obtain descriptive data or specific characters of each genotype [8]. PCR-based molecular markers that are currently widely used for genetic diversity analysis are microsatellites (simple sequence repeat/SSR) because they have several advantages including very informative, high levels of polymorphism and stability, codominance, and ease to apply [9]. Research related to genetic diversity with SSR markers has been carried out to determine the level of genetic diversity of local pigmented rice varieties [10–13]. Morpho-agronomical traits and molecular markers were chosen as the basis for this genetic diversity study, in the hope that more comprehensive information could be obtained regarding the genetic diversity of local pigmented rice from Eastern Indonesia. The use of molecular markers to help identify the genetic diversity of germplasm, especially rice, can help plant breeding programs exploit the potential of local pigmented rice accessions. However, to date, no in-depth research results have been found on the genetic diversity of local varieties of pigmented rice from Eastern Indonesia. This research was conducted to analyze the genetic diversity of 15 accessions of local pigmented rice collected from Eastern Indonesia (Sulawesi and East Nusa Tenggara) in IAARD-ICABIOGRAD Gene Bank based on 33 morpho-agronomical characters and 24 SSR markers.

MATERIALS AND METHODS

Genetic Materials

The genetic materials used in this study included local pigmented rice accessions from Eastern Indonesia, namely Sulawesi Island and East Nusa Tenggara (NTT) Province (Table 1). All accessions of pigmented rice were planted in the greenhouse of Cikeumeuh Experimental Station, Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Indonesian Agency for Agricultural Research and Development (ICABIOGRAD, IAARD) from August to December 2020 during the rainy season. A total of 15 accessions of local pigmented rice were collected from four provinces in Eastern Indonesia, namely NTT (9 accessions), West Sulawesi (3 accessions), South Sulawesi (1 accession), and North Sulawesi (2 accessions). The seeds of these accessions are obtained from the IAARD-ICABIOGRAD Gene Bank.

TABLE 1. Fifteen accessions of local pigmented rice from Eastern Indonesia used in this study.

No.	Accession number	Accession name	Province of origin	No.	Accession number	Accession name	Province of origin
1	05020-20752	Padi K. Hitam	NTT	9	05020-30855	Woja L. Rakot	NTT
2	05020-30480	Ketan Hitam	NTT	10	05020-30779	Pare Tb. Padang	West Sulawesi
3	05020-30836	Woja Rakot	NTT	11	05020-30788	Pare Bae Merah	West Sulawesi
4	05020-30843	Lea	NTT	12	05020-30950	Ba'da (Bae Merah)	West Sulawesi
5	05020-30845	Kartuna	NTT	13	05020-04361	Pulu Palapa	South Sulawesi
6	05020-30846	Laka	NTT	14	05020-06550	Ritgen	North Sulawesi
7	05020-30849	Kosu Ampera	NTT	15	05020-07254	Iden	North Sulawesi
8	05020-30850	Kosu Mite	NTT				

Morpho-Agronomic Traits

The local pigmented rice seeds used in the study were sown in the seedbed and after 21 days after sowing, the seeds were transplanting to a planting pot containing a mixed medium of manure and soil (1:2). Maintenance was carried out by providing NPK compound fertilizer (175 kg/ha) at 1 week after planting (WAP) and urea fertilizer (300 kg/ha) at 3 WAP and 9 WAP. The experiment was designed in a randomized complete block design (RCBD) with three replications. Observations of 33 morpho-agronomical traits consisting of 20 morphological characters and 13 agronomic characters were carried out based on the guidelines of the Standard Evaluation System for Rice adopted from IBGR-IRRI and the Conduct of Test for Distinctness, Uniformity, and Stability for Rice adopted from

UPOV. The morphological characters observed were ligule shape, ligule color, auricle color, flag leaf angle, leaf blade color, basal leaf sheath color, leaf blade pubescence, leaf length, leaf width, awn color, culm internode color, culm angle, diameter of basal internode, panicle type, panicle length, secondary branching of panicle, panicle attitude in relation stem, lemma and palea color, decorticated grain shape, and anthocyanin coloration of keel. Whereas, the agronomic characters observed were tillers number per hill, panicle exertion, culm strength, percentage of filled grains, panicle threshability, plant height, days to heading, days to maturity, panicles number per hill, panicle weight per hill, grain weight per hill, and 100-grain weight.

Molecular Characterization

Molecular characterization was conducted at the Molecular Biology Laboratory, ICABIOGRAD from October to December 2020. A sampling of planting material from each accession was carried out on plants that were 4 weeks. Sampling was taken from each replicate for each accession proportionally and then mixed for genomic DNA extraction using the GenEx Plant Kit. After obtaining the stock DNA solution, the concentration and purity were measured using a NanoDrop2000 Spectrophotometer. DNA quality test was carried out using the electrophoresis technique on 1% agarose gel.

The concentration of DNA used in the PCR process was 10 ng/µl and DNA amplification was carried out with a PCR machine (Thermocycler) with the following program: initial denaturation (95°C for 3 min), followed by 35 denaturation cycles (95°C for 15 sec), annealing (55°C for 15 sec), and elongation (72°C for 1 sec), then followed by final elongation (72°C for 1 min). A total of 24 SSR markers were used to amplify the DNA samples of all local pigmented rice accessions tested in this study (Table 2). The PCR results were then separated using 6% nondenatured polyacrylamide gel in a tank containing 1× Tris-Borate-EDTA (TBE) buffer at 90 volts for 70 min. The gel from the electrophoresis was stained using ethidium bromide solution and visualized on a UV transilluminator using a gel documentation system.

TABLE 2. Twenty-four SSR markers used to amplify the DNA samples of local pigmented rice accessions from Eastern Indonesia.

No.	Markers	Chromosome	SSR motif	No.	Markers	Chromosome	SSR motif
1	RM5	1	(GA)14	13	RM180	7	(ATT)10
2	RM259	1	(CT)17	14	RM11	7	(GA)17
3	RM154	2	(GA)21	15	RM3571	8	(GA)12
4	RM166	2	(T)12	16	RM223	8	(CT)25
5	RM514	3	(AC)12	17	RM105	9	(CCT)6
6	RM282	3	(GA)15	18	RM215	9	(CT)16
7	RM252	4	(CT)19	19	RM474	10	(AT)13
8	RM261	4	C9(CT)8	20	RM5756	10	(AGA)8
9	RM161	5	(AG)20	21	RM144	11	(ATT)11
10	RM413	5	(AG)11	22	RM287	11	(GA)21
11	RM3463	6	(CT)20	23	RM19	12	(ATC)10
12	RM541	6	(TC)16	24	RM277	12	(GA)11

Data Analysis

The quantitative morpho-agronomical data obtained were analyzed by analysis of variance (ANOVA) and followed by Duncan's test at the α = 5% using SAS software. In cluster analysis for combined data, morpho-agronomic character data were arranged in nominal, ordinal, and interval data using Microsoft Excel software. As for the allele scoring data from the GelAnalyzer program, the scoring returns to binary data with code (1) if there is a band and code (0) if there is no band. SSR marker characteristics analysis was carried out to determine the dominant allele frequency, the number of alleles per locus (allele number), gene diversity, and Polymorphism Information Content (PIC) value, the analysis was carried out with PowerMarker software [14]. Furthermore, the combined data from characterization based on morpho-agronomical characters and SSR markers were analyzed using the Gower method and dendrogram visualization with the Divisive method using the PBSTAT-CL program. The number of groups or clusters is determined based on the results of the analysis using the Partitioning Around

Medoids (PAM) method. Based on the PAM analysis, it was found that the best number of clusters in the grouping of all the researched accessions was obtained [15].

RESULTS AND DISCUSSIONS

Variability of Morpho-Agronomical Characters

Variation of Plant Growth Traits

Table 3 presents the average of growth component characters of 15 accessions of local pigmented rice from Eastern Indonesia. Based on the results of the analysis, the characteristics of days to heading and days to maturity of accession Kartuna were the earliest and significantly different from other accessions. Days to maturity is one of the characteristics that many farmers consider, with an early harvest can increase the harvest period [16]. In leaf character, accession Pare Tumbu Padang was the widest leaf and significantly different from other accessions. Meanwhile, accession Woja Rakot had the highest leaf width and diameter of the basal internode. The characters related to leaf area can provide great potential in the formation of assimilating products needed for the formation of productive tillers, panicle initiation, and seed filling in rice plants, thereby affecting yields [17]. Rice leaf area which is determined by the length and width of the leaves will contribute to grain yield because it is the main organ in producing carbohydrates through photosynthesis [18]. Based on the results of the analysis, accession Iden had the highest plant height of 204.90 cm and significantly different from the accession Ketan Hitam with the lowest height of 147.63 cm. In this study, all pigmented rice accessions with plant heights above the ideal rice characteristics of 115–120 cm [19] also had fairly long plant life. This is in good agreement with Yoshida's statement [20] that plant height is closely related to biomass partitioning and plant age, the higher the rice plant is generally followed by long plant life.

TABLE 3. The average of growth component characters in 15 accessions of local pigmented rice from Eastern Indonesia.

Accession name	Days to heading	Days to maturity	Leaf width (cm)	Leaf length (cm)	Diameter of basal internode (cm)	Plant height (cm)
Pulu Palapa	99.0 b	129.7 bc	51.93 i	1.63 fgh	0.94 abcd	166.80 de
Ritgen	111.3 a	147.0 a	64.33 gh	2.17 abc	0.93 abcd	184.00 b
Iden	116.7 a	151.7 a	95.40 ab	2.10 abcd	1.04 ab	204.90 a
Padi Ketan Hitam	80.3 fe	115.0 f	77.97 de	1.50 gh	0.66 e	168.47 cde
Ketan Hitam	93.3 cb	131.3 bc	82.43 cd	1.63 fgh	1.01 abc	147.63 f
Pare Tumbu Padang	99.0 b	133.3 bc	97.07 a	1.73 efg	0.97 abcd	183.13 b
Pare Bae Merah	99.0 b	146.0 a	70.10 fg	1.67 fg	0.91 bcd	179.00 bc
Woja Rakot	87.3 cde	124.3 cde	83.77 cd	2.40 a	1.12 a	184.53 b
Lea	81.0 ef	114.3 f	70.23 gh	2.33 ab	0.91 bcd	163.03 e
Kartuna	63.7 g	102.7 g	64.33 gh	1.53 gh	0.83 cde	157.10 ef
Laka	95.3 bc	135.3 b	83.57 cd	1.77 defg	1.03 ab	198.53 a
Kosu Ampera	83.3 de	116.0 ef	72.27 ef	1.97 cdef	0.81 de	177.67 bcd
Kosu Mite	74.3 f	120.3 def	58.93 h	1.30 h	0.79 de	157.20 ef
Woja Laka Rakot	90.7 bcd	128.7 bcd	77.57 de	2.03 bcde	1.04 ab	181.33 b
Ba'da (Bae Merah)	113.7 a	148.7 a	89.30 bc	2.10 abcd	1.08 ab	184.73 b

Means followed by the same letters in the same column are not significantly different based on Duncan's test at $\alpha = 5\%$.

Variation of Yield Component Traits

The results of the character analysis of the yield components in Tabel 4 showed that accession Ritgen had the highest number of tillers, panicle number, panicle weight per hill, and grain weight per hill, and was significantly different from other accessions. This shows that accession Ritgen has an advantage in terms of yield potential and can be recommended as one of the parents of the cross. The tiller number is an important architectural character for grain yield because the number of tillers per hill can determine the number of panicles per hill and has a direct effect on rice production [21]. The character of each yield component is the sink size that will determine the grain yield [17]. In the character of panicle length and 100-grain weight, accession Laka had the highest panicle length and 100-grain weight and was significantly different from other accessions. The yield components of rice in each accession tested were influenced by the ability of each plant to respond to environmental influences during the growing period. Each accession has a different genetic potential, resulting in different growth and yield variability.

TABLE 4. The average of yield component characters of 15 accessions of local pigmented rice from Eastern Indonesia.

Accession name	Tiller number	Panicle number	Panicle length (cm)	Filled grain (%)	Panicle weight per hill (g)	Grain weight per hill (g)	100-grain weight (g)
Pulu Palapa	30.0 ab	26.3 ab	32.59 de	93.50 a	139.10 ab	127.36 a	2.24 de
Ritgen	31.3 a	28.0 a	29.47 efg	78.30 bcde	143.94 a	131.80 a	2.10 de
Iden	27.0 abc	25.3 ab	33.67 cd	86.35 abc	115.63 abc	106.02 ab	2.13 de
Padi Ketan Hitam	26.3 abc	23.7 abc	27.02 g	73.81 cdef	57.96 ef	49.42 de	1.95 e
Ketan Hitam	27.3 abc	27.3 a	23.75 h	88.6 ab	102.74 abcde	94.83 abcd	2.60 cd
Pare Tumbu Padang	21.0 bcd	20.3 abcd	35.97 bc	78.89 bcde	94.64 bcde	86.75 abcd	2.93 abc
Pare Bae Merah	19.7 cd	16.3 cde	29.39 fg	70.16 ef	61.92 def	56.12 cde	3.25 ab
Woja Rakot	16.7 de	16.0 cde	35.73 bc	77.65 bcde	105.05 abcde	96.90 abcd	2.64 cd
Lea	23.7 abcd	21.0 abcd	31.86 def	85.00 abc	101.27 abcde	95.94 abcd	3.38 a
Kartuna	10.0 e	8.3 e	30.56 ef	71.18 def	25.13 f	22.11 e	3.39 a
Laka	27.0 abc	23.6 abc	40.43 a	63.20 fg	110.26 abcd	100.09 abc	3.41 a
Kosu Ampera	24.3 abcd	20.3 abcd	31.34 def	83.86 abcd	71.69 cdef	63.11 bcde	2.96 abc
Kosu Mite	30.0 ab	27.3 a	30.05 efg	88.60 ab	84.17 cde	74.86 bcd	2.01 e
Woja Laka Rakot	18.3 cde	17.7 bcd	37.27 b	79.20 bcde	70.42 cdef	63.48 bcde	3.03 abc
Ba'da (Bae Merah)	19.0 cd	13.7 de	29.15 fg	51.79 g	28.44 f	23.99 e	2.84 bc

Means followed by the same letters in the same column are not significantly different based on Duncan’s test at $\alpha = 5\%$.

Variations of Morphological Traits

Based on the results presented in Table 5, there are complete variations according to the rice plant descriptor for leaf blade pubescence characters, flag leaf angle, culm angle, culm strength, panicle type, secondary branching of panicles, panicle exertion, and anthocyanin coloration of keel. As for the other characters, there were no complete character variations from the 15 accessions of local pigmented rice used in this study. Additionally, there was no variability for ligule shape and auricle color, i.e. all accessions have a cleft ligule shape and white auricle. It is assumed that the leaf tongue shape of rice cultivars from Indonesia is mostly cleft [22]. This is because until recently there has been no leaf tongue shape in rice cultivars from Indonesia other than cleft. The genetic factors of the qualitative characters are mostly simpler to elucidate than those of quantitative characters because they are only influenced by one or two genes and are not influenced by the growing environment [23].

Variability on other leaf characters was observed on ligule color, leaf blade color, the basal leaf sheath color, and leaf blade pubescence. In general, most of the accessions observed had white ligule color, green leaf blade color, green basal leaf sheath color, medium leaf blade pubescence, and upright flag leaf angle. Leaf morphology is among the important morphological traits to evaluate because it can be observed from the vegetative to generative phase and is a basic study in producing superior rice varieties [24].

In stem characters, there are complete variations according to the rice plant descriptor. In general, most of the accessions had horizontal and descending flag leaf angles, intermediate culm angles, green culm internode color, and strong culm strength. The culm character is one of the important characters in the assembly of high-yielding varieties because it supports high yield potential. The culm functions as a support for plants, a distributor for chemical compounds and water, as well as a food reserve. Strong stems will support high yields because they reduce the risk of the plant falling. In this study, some of the 15 accessions used had strong culm strength characters, namely Ketan Hitam, Pare Tumbu Padang, Pare Bae Merah, Woja Rakot, Lea, Woja Laka Rakot, and Ba'da.

Observations on the characteristics of panicle and grain showed similar results to the characters of leaves and culm, most of them had reasonably high diversity. More than 50.0% of all accessions had intermediate panicle type and weak secondary branching of panicle types. Of the three categories of panicle exertion, as many as 80.0% had the type of panicle exertion well exerted with the characteristics of the panicle attitude The characteristics of panicle threshability as much as 53.3% of the 15 accessions had moderately difficult category and none of the accessions had a difficult category. Neither difficult nor easy panicle threshability is on demand because if the grains are difficult to shed, it will take a long time to separate the grain from the stalk. On the contrary, easy thresability allows grain loss before harvest [25].

TABLE 5. Variations of morphological traits among 15 accessions of local pigmented rice from Eastern Indonesia.

No.	Qualitative characters	Variability and percentage among accessions
1	Ligule shape	1 = acute to acuminate (0%), 2 = cleft (100.0%), 3 = truncated (0%)
2	Ligule color	1 = white/colorless (93.3%), 2 = green (6.7%), 3 = purple lines (0%), 4 = light purple (0%), 5 = purple (0%)
3	Auricle color	1 = white (100.0%), 2 = light green (0%), 3 = purple (0%)
4	Leaf blade color	1 = light green (0%), 2 = green (93.3%), 3 = dark green (0%), 4 = purple tips (6.7%), 5 = purple margins (0%), 6 = purple mixed with green (0%), 7 = purple (0%)
5	Basal leaf sheath color	1 = green (86.7%), 2 = purple lines (13.3%), 3 = light purple (0%), 4 = purple (0%)
6	Leaf blade pubescence	1 = glabrous (6.7%), 2 = intermediate (53.3%), 3 = pubescent (40.0%)
7	Flag leaf angle	1 = erect (6.7%), 3 = intermediate (26.7%), 5 = horizontal (33.3%), 7 = descending (33.3%)
8	Culm angle	1 = erect (6.7%), 3 = intermediate (46.7%), 5 = open (20.0%), 7 = spreading (26.7%)
9	Culm internode color	1 = green (66.7%), 2 = light gold (0%), 3 = purple lines (26.7%), 4 = purple (6.7%)
10	Culm strength	1 = strong (46.7%), 3 = moderately strong (26.7%), 5 = intermediate (20.0%), 7 = weak (6.7%)
11	Panicle type	1 = compact (6.7%), 3 = intermediate (66.7%), 5 = open (26.7%)
12	Secondary branching of panicles	1 = weak (66.7%), 2 = heavy (13.3%), 3 = clustered (20.0%)
13	Panicle attitude in relation to stem	1 = upright (40.0%), 2 = semi-upright (40.0%), 3 = slightly drooping (20.0%), 4 = strongly drooping (0%)
14	Panicle exertion	3 = partly exerted (13.3%), 5 = exerted (6.7%), 9 = well exerted (80.0%)
15	Awn color	0 = awnless (13.3%), 1 = straw (33.3%), 2 = gold (0%), 3 = brown (6.7%), 4 = red (13.3%), 5 = purple (33.3%), 6 = black (0%)
16	Lemma and palea color	0 = straw (20.0%), 1 = gold and gold furrows on straw background (6.7%), 2 = brown spots on straw (0%), 3 = brown furrows on straw (46.7%), 4 = brown (0%), 5 = reddish to light purple (0%), 6 = purple spots on straw (6.7%), 7 = purple furrows on straw (13.3%), 8 = purple (6.7%), 9 = black (0%), 10 = white (0%)
17	Panicle threshability	1 = difficult (0%), 3 = moderately difficult (53.3%), 5 = intermediate (13.3%), 7 = loose (26.7%), 9 = easy (6.7%)
18	Anthocyanin coloration of keel	1 = absent/very weak (20.0%), 3 = weak (20.0%), 5 = medium (26.7%), 7 = strong (33.3%)
19	Decorticated grain shape	1 = round (20.0%), 2 = semiround (40.0%), 3 = half spindle-shape (40.0%), 4 = spindle shape (0%), 5 = long spindel-shape (0%)
20	Decorticated grain color	1 = white (0%), 2 = light brown (0%), 3 = variegated brown (0%), 4 = dark brown (6.7%), 5 = light red (0%), 6 = red (60.0%), 7 = variegated purple (20.0%), 8 = purple (0%) 9 = dark purple/black (13.3%)

The observations on the morphological characters of panicle and grain, showed that there was diversity in the 15 accessions observed. The character of the awn color varies from awnless, straw, brown, red, and purple. The variability was also found in the lemma and palea color, nemely, straw, gold, and gold furrows on straw background, brown furrows on straw, purple spots on straw, purple furrows on straw, and was dominated by brown furrows on straw as much as 46.7% of all accessions. Semiround and half spindle-shape dominated the character of decorticated grain shape. Meanwhile, 60.0% of the 15 accessions had red decorticated grain color, 20.0% variegated purple, and 13.3% had black decorticated grain colors. Anthocyanin coloration of keel variations, decorticated grain shape, and decorticated grain color in the most various accessions of local pigmented rice from Eastern Indonesia is shown in Fig. 1.

FIGURE 1. Anthocyanin color variations in the husk line, rice color, and rice shape in local pigmented rice accessions from Eastern Indonesia.

CHARACTERISTICS OF SSR MARKERS

The results of the analysis of the SSR marker profile showed that all the SSR markers were polymorphic with a total of 117 alleles identified (Table 6). The number of alleles ranged from 3 to 9 alleles. Categorically, the average number of alleles for local pigmented rice accessions from Indonesia tested was 4.87 alleles per locus. The value of gene diversity shows the level of genetic diversity in a population. The index of gene diversity reflecting the degree of polymorphism in this study varied from 0.33 (RM166) to 0.82 (RM 223) with an average of 0.66. Heterozygosity, which is part of the gene diversity index, was observed in 15 accessions of local pigmented rice from Eastern Indonesia tested and identified by two SSR primers.

TABLE 6. Variations of morphological traits among 15 accessions of local pigmented rice from Eastern Indonesia.

Locus	Number of alleles	Major allele frequency	Gene diversity	Heterozygosity	PIC
RM 161	6	0.33	0.77	0.00	0.74
RM 166	3	0.80	0.34	0.00	0.31
RM 180	5	0.53	0.65	0.00	0.61
RM 223	7	0.27	0.82	0.47	0.80
RM 252	9	0.40	0.79	0.00	0.77
RM 261	3	0.60	0.56	0.00	0.50
RM 277	4	0.47	0.67	0.00	0.61
RM 282	5	0.53	0.66	0.00	0.62
RM 413	5	0.33	0.73	0.00	0.68
RM 541	8	0.33	0.82	0.00	0.80
RM 3463	3	0.73	0.42	0.00	0.37
RM 3571	4	0.50	0.62	0.07	0.55
RM 5756	3	0.53	0.59	0.00	0.51
RM 5	5	0.40	0.74	0.00	0.70
RM 11	6	0.40	0.76	0.00	0.72
RM 19	3	0.67	0.48	0.00	0.41
RM 105	5	0.47	0.69	0.00	0.65
RM 144	4	0.40	0.68	0.00	0.62
RM 154	5	0.33	0.74	0.00	0.69
RM 215	3	0.53	0.60	0.00	0.54
RM 259	6	0.40	0.74	0.00	0.70
RM 287	6	0.33	0.75	0.00	0.71
RM 474	5	0.33	0.74	0.00	0.69
RM 514	4	0.67	0.52	0.00	0.48
Mean	4.88	0.47	0.66	0.02	0.62

PIC = Polymorphic Information Content.

Heterozygosity was indicated by two SSR markers, namely RM 223 and RM 3571. Molecular markers that are good for genetic diversity studies have high PIC values. The PIC value is a description of the ability of a molecular marker to produce polymorphic alleles [26]. The PIC value is needed to select a marker that can distinguish between the local pigmented rice accessions tested. Of the 24 SSR markers used, 3 SSR markers have a PIC value >0.75, namely the RM 223, RM 252, and RM 541 markers, with the highest PIC value being the RM 223 marker which is 0.80. The average PIC value is 0.62. There is a tendency that the higher the PIC value, the more alleles detected.

The high mean PIC value is an indication of the high genetic diversity of the germplasm tested. The PIC value of molecular markers can be used as a basis for selecting differentiating markers between genotypes. The PIC value is determined not only by the number of alleles, but also by the frequency of each allele in the set of tested genotypes [27]. The average PIC value in this study was similar to that of the aromatic rice germplasm study observed by Aljumaili *et al*. [28] with an average PIC value of 0.63. All markers used in this study are quite informative and show their suitability for differentiating accessions of local pigmented rice so that they have the potential to be used to explore the genetic diversity of other local pigmented rice accessions in Indonesia.

Principal Coordinate Analysis (PCoA)

Genetic diversity analysis in germplasm is important for identifying potentially valuable genetic materials [29]. Multivariate statistical techniques could be used to analyze multiple measurements on each individual studied simultaneously and widely used in the analysis of genetic diversity irrespective of whether it is based on morphological or molecular marker [30]. The pattern of grouping pigmented rice with local accessions from Eastern Indonesia based on morpho-agronomical characters and SSR markers is shown in the results of the Principal Coordinate Analysis (PCoA). The main coordinate analysis is an analysis used to determine the grouping pattern between accessions based on the similarity of characters through dimensional simplification [31]. The PCoA is very useful for analyzing genetic diversity among accessions in germplasm [32]. In this study, the cluster analysis method was carried out using PAM method based on Gower's dissimilarity coefficients among accessions. Fig. 2 shows the relative positions of 15 accessions of local pigmented rice from Eastern Indonesia as a result of the main coordinate analysis.

The main coordinate analysis generated three groups which differentiated each other based on different colors, were obtained (Fig. 2). The first group of combined data analysis results consisting of nine accessions, namely Pare Tumbu Padang, Pare Bae Merah, Woja Rakot, Lea, Kartuna, Laka, Kosu Ampera, Woja Laka Rakot, and Ba'da (Bae Merah). Of the 9 accessions, 3 of them came from Sulawesi Island, and 6 others came from NTT Province. Of all the accessions in group I, 7 accessions were characterized by red decorticated grain color and 2 accessions with variegated purple.

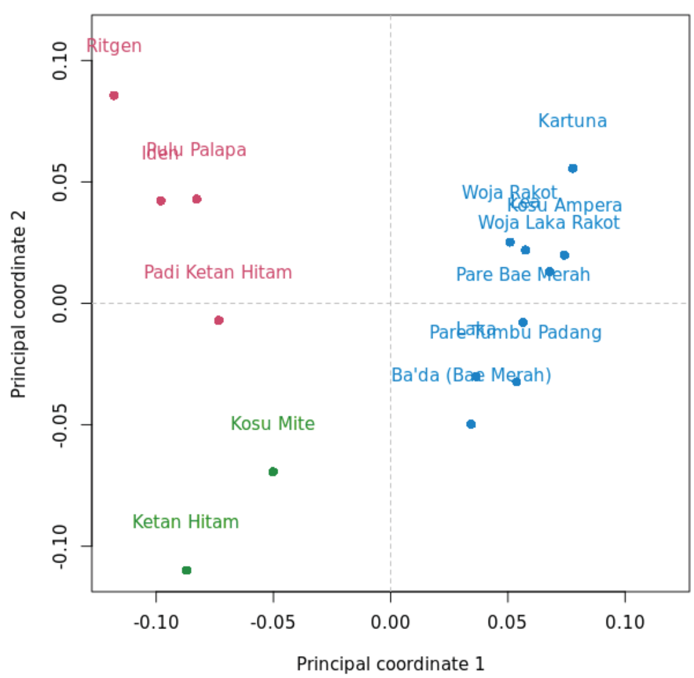

FIGURE 2. The relative position of 15 accessions of local pigmented rice from Eastern Indonesia based on 33 morpho-agronomical characters and 24 SSR markers on the results of PCoA analysis.

In group II there were two accessions, namely accessions Ketan Hitam and Kosu Mite, came from the same province, namely NTT. The similarity between the two accessions is that they are decorticated grain color with black color and half spindle shape. In group III, there were four accessions, namely Pulu Palapa, Ritgen, Iden, and Padi Ketan Hitam. The four accessions come from different provinces and also have different decorticated grain colors.

The grouping of accessions based on regional origin and decorticated grain color in this study were not entirely separated clearly in groups I and III, but accessions with black decorticated grain color tended to be grouped in group II and for red decorticated grain color they tended to be clustered in group I. The grouping of local pigmented rice accessions that have not been separated based on the color of decorticated grain and the area of origin is thought to be because genetic diversity is influenced by the origin of the parents. Accessions with a close genetic distance can come from closely related parents, while accessions with a relatively high genetic distance are thought to come from parents who are far from kinship.

A similar study reported that in the genetic diversity of rice germplasm from Java, not all rice accessions from this island were grouped according to their region of origin [33]. However, different research results were reported that the genetic diversity of aromatic rice, where all aromatic rice genotypes tested for genetic diversity are grouped according to their geographical conditions of origin [28]. The distribution of local rice varieties into different groups revealed that genetic diversity existed among these local rice varieties. The results of this PCoA analysis can provide initial information regarding genetic diversity in local pigmented rice from Eastern Indonesia if it is to be used as genetic material for plant breeding.

Phylogenetic Analysis

The phylogenetic analysis aims to identify the genetic distance between accessions [34]. Phylogenetic analysis was performed using the Divisive method based on Gower's dissimilarity coefficients. The results of phylogenetic analysis based on the morpho-agronomical traits and SSR markers showed grouping of 15 accessions of local pigmented rice variety into three main groups (Fig. 3). These results are consistent with the result of the PCoA analysis. The dendrogram visualization shows that at a cophenetic distance of 0.22 there were three groups, the first group consisting of 9 accessions, the second group composing 2 accessions, and the third group consisting of 4 accessions. At this distance, it shows that the 15 accessions still have a relatively close kinship. This is presumably because the number of accessions used is still small and originates from the same region, namely Sulawesi Island and NTT Province.

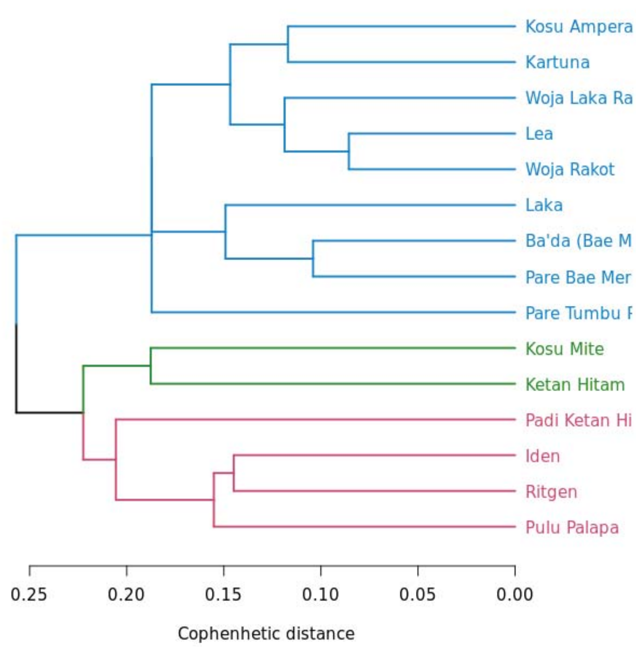

FIGURE 3. Dendrogram of genetic diversity of 15 accessions of local pigmented rice from Eastern Indonesia using 33 morpho-agronomical traits and 24 SSR markers.

Based on the combined characters, some accessions have distant kinship relationships and other accessions are closely related. This can be seen from the coefficient of genetic diversity in Fig. 3, which shows the coefficient of genetic diversity is about 80%. The coefficient of genetic diversity based on 33 morpho-agronomic characters and 24 SSR markers indicated that accessions Woja Rakot and Lea had a close relationship. Therefore, these two accessions were not recommended to be crossbred because it could cause the phenomenon of inbreeding depression.

Meanwhile, accessions Ketan Hitam and Kartuna have a distant relationship, as presented in the similarity matrix heatmap in Fig. 4.

The combination of the two data sets, namely morpho-agronomic data and SSR markers, is expected to provide more comprehensive information when compared to using only one type of data set. The results of molecular characterization would be beneficial in the efficiency of plant breeding programs [35]. Information regarding the kinship relationship between accessions of local pigmented rice from Eastern Indonesia can be used to develop a strategy for breeding pigmented rice plants in the future. To increase heterozygosity, crosses can be done in accessions that have a tremendous genetic distance. A greater number of accessions, morpho-agronomic characters were observed, and SSR markers can be used to obtain better germplasm grouping results.

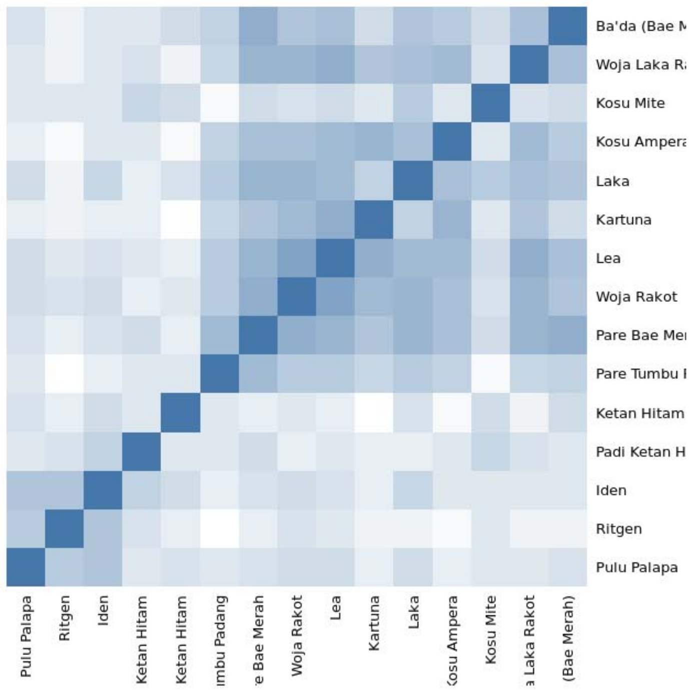

FIGURE 4. Similarity matrix heatmap of 15 accessions of local pigmented rice from Eastern Indonesia.

CONCLUSION

The genetic relationship of 15 accessions of local pigmented rice from Sulawesi Island and NTT Province was able to be assessed using 33 morpho-agronomical characters and 24 SSR markers. The morpho-agronomic characters of all observed accessions varied both in morphological and agronomical characters. The similarity coefficient between accessions based on morpho-agronomic characters and SSR markers was relatively high, around 80%. The results of PCoA analysis and phylogenetic analysis based on combined data analysis formed three different groups at a cophenetic distance of 0.22. The first group consists of accessions Pare Tumbu Padang, Pare Bae Merah, Woja Rakot, Lea, Kartuna, Laka, Kosu Ampera, Woja Laka Rakot, and Ba'da (Bae Merah), the second group consists of accessions Ketan Hitam and Kosu Mite, and the third group consisted of accessions Pulu Palapa, Ritgen, Iden, and Padi Ketan Hitam. Overall, the yield components of rice in each accession tested were influenced by the ability of each plant to respond to environmental influences during the growing period. Each accession has a different genetic potential, resulting in different growth and yield variability.

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