IOP Conference Series: Earth and Environmental Science

Table of contents

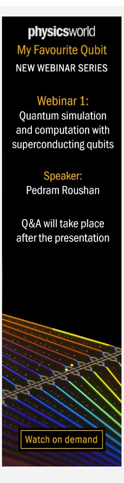
Volume 1255
2023

Previous issue Next issue

The International Conference and Workshop in conjuction with the 8th Indonesia Biotechnology Conference (IBC) 16/05/2023 - 18/05/2023 Bali, Indonesia

Accepted papers received: 29 September 2023
Published online: 21 November 2023

JOURNAL LINKS
Journal home
Journal scope
Information for organizers
Information for authors
Contact us
Reprint services from Curran Associates



Open all abstracts

Preface

OPEN ACCESS 011001
Preface: The International Conference and Workshop in conjunction with the 8th Indonesia Biotechnology Conference (IBC)
+ Open abstract View article PDF

OPEN ACCESS 011002
Peer Review Statement
+ Open abstract View article PDF

Biodiversity for Agriculture and Health.

OPEN ACCESS 012001
Diversity of predators and population *Spodoptera frugiperda* J.E Smith by application botanical insecticide and intercropping cover crops
E Sulastri, S Sjam and Sulaeha
+ Open abstract View article PDF

OPEN ACCESS 012002
Agronomic performance, yield and seed production of some new superior varieties using integrated crop management approach in tabanan district, Bali province
I B K Suastika, I K Kariada, N M Delly Resiani and I M Budiartana
+ Open abstract View article PDF

OPEN ACCESS 012003
The role of rhizosphere microbes as phosphate solubilizing bio fertilizers in shallot: a review
A E Marpaung, D N Susilowati, G A Sopha, D R Siagian, S S Girsang, R Tarigan, I S Marpaung, T F Silitonga, T Sabrina, A Rauf *et al*
+ Open abstract View article PDF

OPEN ACCESS 012004
The effect of N, P and K organic substances enhancers on fruit waste liquid organic fertilizer on tomato plants
L Walunguru and M M Kaka Mone
+ Open abstract View article PDF

OPEN ACCESS 012005
The effect of AB mix fertilizer concentration on the yield of Marigold (*Tagetes erecta*) under hydroponic condition
Y J Nudin, L Walunguru and N D Lussy
+ Open abstract View article PDF

OPEN ACCESS 012006
The Use of rhizobia and vesicular-arbuscular mycorrhiza to improve drought resistance of Kudzu (*Pueraria phaseoloides* (Roxb.)Benth.) in coastal land
N Triwahyuningsih and T B Kusmiyarti
+ Open abstract View article PDF

OPEN ACCESS 012007
Eco-enzyme enhanced the growth of rice cultivars
M R Defiani and I A Astarini
+ Open abstract View article PDF

OPEN ACCESS 012008
Effect of application liquid organic fertilizer Bio3 with some concentrations to carrot (*Daucus carota* L.) yield
E H A Juwaningsih, C Pandjaitan, I K Sudarma and Y I S Baok
+ Open abstract View article PDF

OPEN ACCESS 012009
The role of combination insectary plants and botanical pesticides in increasing arthropod populations of natural enemies and suppressing populations of *s. frugiperda* in corn
Muliadi, S Sjam and S Thamrin
+ Open abstract View article PDF

OPEN ACCESS 012010
Host range of three isolates of papaya ringspot virus (PRSV) found in North Sumatera and West Java
Riska, T Budiyanti, Jumjunidang and E D Husada
+ Open abstract View article PDF

OPEN ACCESS 012011
Damage Caused by *Spodoptera frugiperda* J.E Smith on Corn in Climate Zones in South Sulawesi, Indonesia
A Vebryanti, I D Daud and N E Dungga
+ Open abstract View article PDF

OPEN ACCESS 012012
Variability and selection of tomato *Sletr1-2* mutants backcross population to improve yield and fruit quality
I R Saadah, G Wiguna, Anas, S Mubarok, K K Hamdani and R P Soedomo
+ Open abstract View article PDF

OPEN ACCESS 012013
Morphological characterization and screening of Impatiens wild species for heat tolerance
S Kartikaningrum, Mawaddah, D Pramanik, M Dewanti, DS Badriah, H Shintiavira, E Diningsih, Hanudin, W Nuryani, D Sihombing *et al*
+ Open abstract View article PDF

OPEN ACCESS 012014
Morphological characters and yields of five Vanilla (*Vanilla planifolia* Andrews) clones in Bali
R Heryanto, E Hadipoentyanti, S Wahyuni and L Udarno
+ Open abstract View article PDF

OPEN ACCESS 012015
Evaluation of Hard Seed in Indonesia Local Mungbean (*Vigna radiata* L.)
H Afza, E R Palupi, S Ilyas and L Herlina
+ Open abstract View article PDF

OPEN ACCESS 012016
Total phenolic and flavonoid contents of *Aphanamixis polystachya* (Wall.) R.Parker leaf extract and its potential as antioxidant and inhibitor of α-glucosidase
I P A H Wibawa, M Hanafi, A S Li'aini, A Rahayu, I N Lugrayasa, V M Butardo and P J Mahon
+ Open abstract View article PDF

OPEN ACCESS 012017
Isolation of potential Zn solubilizing bacteria from corn rhizosphere
A Irmadamayanti, M Jayadi and K Mantja
+ Open abstract View article PDF

OPEN ACCESS 012018
Ex vitro shoot induction of rodent tuber (*Typhonium flagelliforme*) with benzyl amino purine and naphthalene acetic acid
L Hardiany, Y S A Fauzan, Pramono, S Rosmalawati, L Novita, I F Hindaningrum and F R Mira
+ Open abstract View article PDF

OPEN ACCESS 012019
Tissue Culture of Four Varieties of Date Palm Grown in Indonesia
M M Sinta, R T Saptari, I Riyadi and Sumaryono
+ Open abstract View article PDF

OPEN ACCESS 012020
Effectivity of liquid organic fertilizer on production of pakcoy (*Brassica rapa* L.)
H Rahmi, H R Fardany, F M Bayfurqon and J H Jaman
+ Open abstract View article PDF

OPEN ACCESS 012021
The potential of new hybrid Chrysanthemum as germplasm resource
R Kurniati, Suryawati, D W Utami, M Pramayufdy, R D Purwati, J Mulyono, M Susilowati, Supenti and R Syafarina
+ Open abstract View article PDF

Biodiversity for Environment and Tourism

OPEN ACCESS 012022
Effect of hatchery and mariculture activity at the northwest coastal area of Bali on water quality and plankton community
B Slamet and C Kardi
+ Open abstract View article PDF

OPEN ACCESS 012023
Recollection and conservation of *Vatica javanica* subsp. *scaphifolia*, a critically endangered endemic tree species in Southeastern Borneo
E Primananda, B A Pratama, R Susanti, Y Wibisono, A Hamidi, D S Rinandio and I Robiansyah
+ Open abstract View article PDF

OPEN ACCESS 012024
Urgent propagation of the endemic and possibly extinct in the wild tree *Cassine koordersii* (Celastraceae)
P I Pratiwi, E Rahma, A Astuti, D Hastuti, J E R Rumbiak, A H Widjaya, I Robiansyah and E Primananda
+ Open abstract View article PDF

OPEN ACCESS 012025
The Influence of Activation Technique on the Adsorption of Coconut Coir-Activated Carbon in Four Potentially Dangerous Liquids
H S Wibisono, N Hastuti, D A Indrawan, E N Aini, G Pari and D Hermawan
+ Open abstract View article PDF

OPEN ACCESS 012026
In vitro culture of wild *Vanda tricolor* Lindley seeds from Kintamani, Bali, as initial step for conservation
P E P Gayatri, I A Astarini and M Pharmawati
+ Open abstract View article PDF

OPEN ACCESS 012027
Mangroves species diversity and their use as medicinal plants by coastal communities of Pesawaran Lampung
Duryat, Rodiani and T Maryono
+ Open abstract View article PDF

OPEN ACCESS 012028
Biodiversity, community structure and potential uses of seaweeds and seagrass in transition zone of Taka Bonerate Kepulauan Selayar biosphere reserve, Indonesia
T M Setia, H S Fadhillah, P Wulandari, S Handayani, S Sainal, R A Praptiwi and J Sugardjito
+ Open abstract View article PDF

OPEN ACCESS 012029
Creation of ten new variety of *Begonia* through interspecific Hybridization: A Study from Bali Botanic Garden's Collection
E Hendriyani, I M Ardaka, I N Lugrayasa, T Warseno and H M Siregar
+ Open abstract View article PDF

OPEN ACCESS 012030
Diversity, distribution of the ginger family (Zingiberaceae) in West Sumatra based on herbarium specimens and its potency for genetic resources essential oil
N Rahmi, Nurainas and Syamsuardi
+ Open abstract View article PDF

Biodiversity and Traditional knowledge and Socio-Cultural Aspects

OPEN ACCESS 012031
The sustaining agro-biodiversity through topworking approach: a case of local superior durian conservation on Mount Arjuna
S Purnama, L Anggraeni and T Zubaidi
+ Open abstract View article PDF

OPEN ACCESS 012032
The effect of liquid bio-organic fertiizer on the productivity of ironwood *(Eusideroxylon zwageri)* leaf biomass
A N Laksmita, D M Salam, A Fernandes, R Maharani and S H Saputra
+ Open abstract View article PDF

OPEN ACCESS 012033
Plant diversity and its potential in Sumenep, East Java, Indonesia
S Susiarti, Rugayah, P Oryzanti, Ismawati and R A Destryana
+ Open abstract View article PDF

OPEN ACCESS 012034
Biodiversity and indigenous knowledge on orchid conservation: *Study at a community in Yogyakarta*
L Nuraini, Hastangka and Y Kristiawan
+ Open abstract View article PDF

OPEN ACCESS 012035
Ethnobotanical study of *Zingiber album* Nurainas, an endemic ginger from Sumatra
F Syafira, Nurainas and Syamsuardi
+ Open abstract View article PDF

OPEN ACCESS 012036
Indonesian threatened Zingiberaceae: Exploring their potential traditional and modern uses
M B Atmaja, P S Andila, G A E Oktavia and H Merriansyah
+ Open abstract View article PDF

OPEN ACCESS 012037
Utilization of various honey, fruits, and vegetables to increase hemoglobin levels in pregnant women with anemia in Indonesia
R Widowati
+ Open abstract View article PDF

Biotechnology and Biodiversity Use

OPEN ACCESS 012038
Pitcher formation of *Nepenthes ampullaria* and *Nepenthes rafflesiana* on modified *in vitro* media
Y Isnaini and Y Novitasari
+ Open abstract View article PDF

OPEN ACCESS 012039
Profiling of *HSPB9* gene sequences in Donggala (*Bos indicus*) and Polled Bali (*Bos javanicus*) cattle breeds of Indonesia
H Hasbi, W P B Putra, T Maulana, P P Agung, S Said, H Sonjaya, S Baco, S Gustina, Y Duma, M Tanari *et al*
+ Open abstract View article PDF

OPEN ACCESS 012040
The evaluation of agronomic characters and artemisinin content of EMS-mutated artemisia lines
R Purnamaningsih, A Isnawati and M Masjkur
+ Open abstract View article PDF

OPEN ACCESS 012041
Dendrobium sp. subcultures with instant Vacin Went media against many concentrations of fruit waste liquid organic fertilizer plus
E H A Juwaningsih, A Hasan and C Pandjaitan
+ Open abstract View article PDF

OPEN ACCESS 012042
Somatic embryo formation, plantlet vigorous and greenhouse preliminary test of garlic bulblet obtained through somatic embryogenesis
Y Supriyati, I R Tambunan, Mastur, J Tamami, A A A Azizi, Y Darsa and P Yufdi
+ Open abstract View article PDF

OPEN ACCESS 012043
Immobilization microbial cellulase and lipase as bio-catalyst agent using maltodextrin for biofuel synthesis
A C Djohan, B Subiyanto and A Thontowi
+ Open abstract View article PDF

OPEN ACCESS 012044
Segregation of molecular markers associated with *Bph3* gene in BC_5F_2 population derived from Ciherang rice variety as the recipient parent
M Yunus, A Dadang, Slamet, A Warsun, D Satyawan and Chaerani
+ Open abstract View article PDF

OPEN ACCESS 012045
In vitro multiplication of *Pothos tener* shoots
R Yunita, M F I Nugraha, L Sari and M A L Rajamuddin
+ Open abstract View article PDF

OPEN ACCESS 012046
Metabolite profiling of the medicinal herb *Phyllanthus niruri* L. under drought stress
W Nawfetrias, E Nurhangga, R Reninta, S Chotimah, I S Bidara, D Maretta, L Devy, R R Esyanti and A Faizal
+ Open abstract View article PDF

OPEN ACCESS 012047
Effect of various LED light on the growth and reproductive of *in vitro*-derived Indonesian chili pepper (*Capsicum annum*) Kopay and Laris varieties
Y Sulistyowati, Wahyuni, NS Hartati, J Kim, E Cho, M Seskar, I Raskin and E Sudarmonowati
+ Open abstract View article PDF

OPEN ACCESS 012048
SSR diversity on rice landraces collected from Yogyakarta Province
H Kurniawan, N Hidayatun, Kristamtini, S Widyayanti and A Risliawati
+ Open abstract View article PDF

OPEN ACCESS 012049
Tobacco resistance gene expression levels in response to the infection of *Phytophthora nicotianae*
J Sukweenadhi, C V Christian, J Setiabudi and W D Savitri
+ Open abstract View article PDF

OPEN ACCESS 012050
Autoinduction expression of Bst DNA polymerase using lac operon-controlled expression systems in *Escherichia coli*
F A Laksmi, I Nuryana, C Vivid and M I Hadi
+ Open abstract View article PDF

OPEN ACCESS 012051
Transcriptome analysis of maize explant for anther culture: comparison of three different RNA extraction methods
M Syafii, S Fatimah, M H Rozik, D Purwoko and S Zulaeha
+ Open abstract View article PDF

OPEN ACCESS 012052
Comparison of three different RNA extraction kits for transcriptome analysis of Indonesian rice (*Oryza sativa* L) explant for anther culture
S Fatimah, D Purwoko, I R A Rosyid, S Zulaeha and M Syafii
+ Open abstract View article PDF

OPEN ACCESS 012053
Embryogenic callus induction of Indonesian Cassava (Menti and Adira 4) on different picloram concentrations
A A A Azizi, N Rahman, N S Hartati, S Koerniati, B R Hastilestari, D Sukmadjaja and Witjaksono
+ Open abstract View article PDF

OPEN ACCESS 012054
Textile dye decolourisation of yeast isolated from *Tuak* starter culture
S Setiono, T Sophiano, JS Purnomo, H Victor and R Pinontoan
+ Open abstract View article PDF

OPEN ACCESS 012055
In-silico Gene Editing of *LCYB* in *Musa acuminata* and Its Functional Analysis Related to Lycopene Beta-Cyclase Pathway
M H Fendiyanto, D J Maysha and B R Hastilestari
+ Open abstract View article PDF

OPEN ACCESS 012056
Callus stage and morphology affect the DNA yield on the DNA extraction from the sugarcane *in vitro* callus
A A Aksa, Y Setiawati, H Minarsih, I Riyadi, M E R B Prasetyo, M M Sinta, Sumaryono and R T Saptari
+ Open abstract View article PDF

OPEN ACCESS 012057
Profiles of Semi-Polar Metabolites from Leaves of *In Vitro*- Derived Plants of Indonesian Pepper Varieties (*Capsicum annuum*) after Gamma Irradiation Treatments
Wahyuni, N Sri Hartati, Yuli Sulistyowati, Ambar Y Perdani, Wasiatur Roziqoh, Renalison Farias-Pereira, Xiaoyang Su, Enny Sudarmonowati and Ilya Raskin
+ Open abstract View article PDF

OPEN ACCESS 012058
Transformation of CRISPR/Cas9 expression construct to enhance saturated fatty acid synthesis in oil palm for biofuel production
H Minarsih, G W Permatasari, Y Setiawati, L D Mardhika, R T Saptari, I Riyadi, A A Aksa and M M Sinta
+ Open abstract View article PDF

OPEN ACCESS 012059
Biomolecular mycorrhizal diversity in *Azadirechta excelsa* (Jack) Jacobs roots in habitats with different altitude
G Anwar, Sipriyadi and C L Ketaren
+ Open abstract View article PDF

OPEN ACCESS 012060
Application of combination of auxins in callus induction and proliferation of shallot cv Trisula and Sumenep through *in vitro* technique
S Rahayu, R Purnamaningsih, A Husni, Chaerani and R Rosliani
+ Open abstract View article PDF

OPEN ACCESS 012061
Fractional inhibitory concentration index of the allicin and flavonoids combinations against *Ganoderma boninense*
C A Yusup, D D Eris, M G Pradana, A S Mulyatni and H Widiastuti
+ Open abstract View article PDF

OPEN ACCESS 012062
Exploration and macroscopic characterization of microbiomes (fungi and bacteria) on anthracnose-Infected chili plants in lowlands fields of Deli Serdang North Sumatra
R Tarigan, C Hermanto, Lisnawita, I Safni, B K Udiarto, A Hasim, W Setiawati, A E Marpaung, A Sebayang, Riska *et al*
+ Open abstract View article PDF

OPEN ACCESS 012063
Determination of Marker Compounds of *Senna alata* from different location in East Kalimantan, Indonesia
D M Salam, A N Laksmita, A Fernandes, R Maharani and S H Saputro
+ Open abstract View article PDF

Advanced Biotechnology and Human Health

OPEN ACCESS 012064
Alcohol concentration from fermentation and distillation of Palm sap (*Arenga pinnata*) in North Halmahera, Indonesia
Y Anwar, C Puspitasari and W Fatriasari
+ Open abstract View article PDF

OPEN ACCESS 012065
Lyoprotectants for *Lactobacillus brevis* B144 and their capability to maintain the expression of surface layer protein
R D Pratiwi and S Zanjabila
+ Open abstract View article PDF

OPEN ACCESS 012066
Development of Polymerase Chain Reaction assay protocol for assessment *Salmonella* sp. in cow raw milk
M Pertiwi-Koentjoro, A Dilla, M Taufiq-Hidayat and E Nugroho-Prasetyo
+ Open abstract View article PDF

OPEN ACCESS 012067
The nano mixture of ethanol extract ketumbar (*Coriandrum sativum* L.) seed and putri malu (*Mimosa pudica* L.) leaves potentially reduced blood glucose levels in hyperglycemia wistar rat
S Wahjuni, N M Puspawati and I B P Manuaba
+ Open abstract View article PDF

OPEN ACCESS 012068
Effects of Temperature Shock on Viability and Stress-Related Gene Expression in *Pediococcus acidilactici*, a Probiotic Lactic Acid Bacteria
I Jonathan, P V P Devanthi, A G A Arham, A A Crystalia, C L S Ying and I T Pramanda
+ Open abstract View article PDF

OPEN ACCESS 012069
Bioprospecting antiobesity and anticholesterol activity of ethanolic extract of *Clidemia hirta* leaves grown at Merapi Mountain National Park in a high fat diet-induced obese Swiss Webster mice
C N Prima, S S Widhiastuti, A Nurwijayanto and I S Arsiningtyas
+ Open abstract View article PDF

OPEN ACCESS 012070
Seven types of phenolic acid and inhibitory activity for protein tyrosine phosphatase 1B (PTP1B) from the stem of *Sandoricum koetjape*
S Saadah, SM Tulandi and RA Rohman
+ Open abstract View article PDF

IOP Publishing © Copyright 2023 IOP Publishing Terms & conditions Disclaimer Privacy & cookie policy

This site uses cookies. By continuing to use this site you agree to our use of cookies.

PAPER • **OPEN ACCESS**

Preface: The International Conference and Workshop in conjunction with the 8th Indonesia Biotechnology Conference (IBC)

To cite this article: 2023 *IOP Conf. Ser.: Earth Environ. Sci.* **1255** 011001

View the article online for updates and enhancements.

You may also like

- Preface
- Challenges and Opportunities in Establishing China Infrastructure for Big Biodiversity Data
  Li Zhu and Keping Ma
- Development of Aceh biodiversity information system
  M Subianto, R P F Afidh and E Harnelly

This content was downloaded from IP address 182.253.116.215 on 24/11/2023 at 17:20

Preface: The International Conference and Workshop in conjunction with the 8th Indonesia Biotechnology Conference (IBC)

The International Conference and Workshop in conjunction with the 8th Indonesia Biotechnology Conference (IBC), which was the first hybrid event held by the Indonesian Biotechnology Consortium, Universitas Nasional (UNAS), in collaboration with National Research and Innovation Agency of Indonesia (BRIN) and Rutgers – The State University of New Jersey USA was held on 16-18 May 2023 at the Prime Plaza Hotel Sanur Bali. The conference is dedicated to providing a forum to build network, exchange ideas about bioscience and biotechnology for addressing the challenges in sustainable developments, especially in Bali – Indonesia, as well as to inspire young academics, scientists and students who are interested in biotechnology (including the new omics technology), biosciences and bioengineering and integrated approaches, and encourage ideas dissemination in these fields.

The joint conference`s theme was "Crossroad of Biodiversity, Biotechnology and Health for Enhancing Sustainable Development" to embrace broader ideas and inputs from academics, practitioners from various scientific backgrounds including economics, engineering, politics, culture, social and humanities sciences, as well as to encourage participation and inputs from policy makers in government bodies who are expected to be able to produce integrated solutions to the current biodiversity challenges. Besides, this conference was a stepping stone to integrate some approaches from various disciplines and also an opportunity to build a network and joint research between institutions for biodiversity conservation as an asset for developing sciences and supporting sustainable development.

The objectives of the conference were: 1) to bring together participants, experts and stakeholders to examine ideas and solutions for maintaining, caring and protecting biodiversity for future sustainable development for human beings, especially in Indonesia, 2) to integrate ideas and inputs from academics from various discipline background to achieve prosperous sustainable biodiversity, 3) to provide a chance of networking between academics and practitioners dealing with biodiversity, 4) to exchange research results on sustainable use as well as conservation of biodiversity, 5) to increase capacity building of young researchers in exploring biodiversity for human health and wellness, and 6) to broaden networking of the participants and to produce a joint research.

The benefit of this conference was creating synergy between national and international academics and institutions with various discipline backgrounds, government bodies, and stakeholders dealing with biodiversity for supporting sustainable development and future community prosperity. The conference and workshop are also a premier interdisciplinary forum for life scientists, engineers, and practitioners to present their latest research results, ideas, developments, and applications in relevant areas.

The joint conference was attended by academics, researchers and practitioners within broad areas of science and technology such as agriculture, forestry, biotechnology and environment, medicine, biology, veterinary, and social sciences. 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 in IOP Proceeding Conference Series.

This two-day conference on 16-17 May 2023 featured research presentations and was closed with discussions with experts and participants to sum up the common conclusion. This occasion was followed by a field visit to Eka Karya Botanic Garden in Bedugul Bali and a workshop for selected participants on 18 May 2023. The first day of the conference consisted of three keynote speakers' around 25 minutes presentations and three plenary sessions by invited speakers. The second day was organized for a plenary session and parallel

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd

sessions which were divided into five topics: (a) Biodiversity for agriculture and health, (b) Biodiversity for environment and tourism, (c) Biodiversity and traditional knowledge and socio-cultural aspects, (d) Biotechnology and biodiversity use, (e) Advanced biotechnology and human health. The invited speakers in the plenary session have a presentation for 30 minutes. The keynote and invited speakers are listed below:

Keynote speakers:
1. Prof. James E. Simon (Rutgers – The State University, USA)
2. Prof. Bambang Prasetya (Indonesian Biotechnology Consortium (IBC), National Commission on Biosafety, Indonesia)
3. Drh. Indra Exploitasia Semiawan M.Si (Ministry of Environment and Forestry, Indonesia)

Invited speakers:
1. Prof. Vyacheslav Dushenkov (Rutgers University, USA)
2. Prof. Saidbek Satorov (Medical-Social Institute of Tajikistan, Tajikistan)
3. Dr. Sulhia Mavlonazarova (Avicenna Tajik State Medical University, Tajikistan)
4. Prof. Ilya Raskin (Rutgers University, USA)
5. Prof. Ernawati Sinaga (Universitas Nasional, Indonesia)
6. Dr. Masteria Yunovilsa Putra (National Research and Innovation Agency/BRIN, Indonesia)
7. Prof. Phillip J. Brantley (Louisiana State University, USA)
8. Assoc. Prof. Paula Rhode Brantley (Louisiana State University, USA)
9. Prof. Heather Zwickey (National University of Natural Science, USA)
10. Prof. Dr. Erin Vogel (Rutgers University, USA)
11. Prof. Hiroshi Kamitakahara (Kyoto University, Japan)
12. Prof. Enny Sudarmonowati (National Research and Innovation Agency/BRIN, Indonesia)
13. Assoc. Prof. Rong Di (Rutgers University, USA)
14. Dr. Inez S. Loedin (International Rice Research Institute, Philippines)
15. Prof. I Gede Putu Wirawan (Udayana University, Indonesia)

We would like to express our sincere gratitude to all speakers, presenters, and all participants for their contributions to this conference and workshop. Special thanks to the Management of Eka Karya Botanic Garden BRIN - Bedugul Bali for facilitating samples for practical health-related property analysis from its plant collections during the workshop. We would also like to express our appreciation for the generosity of our sponsors who supported this conference: PT. Hartech Indonesia, PT. Syngenta Indonesia, Croplife Indonesia, PT. Elokarsa Utama, PT. ITS Indonesia Science, PT. Indotech, PT. Indolab, PT. Corteva and PT. Merck Tbk. This conference is also supported by Asia Pacific Network for Global Change Research (APN) and National Institutes of Health (NIH). Lastly, special thanks to all steering committee, reviewers, and committee members for their great work and contributions to the conference, publication, and workshop.

List of committees:
Steering Committee:
1. Ir. Mastur, MSi., Ph.D. (Indonesian Biotechnology Consortium / IBC)
2. Prof. Dr. Bambang Prasetya (Indonesian Biotechnology Consortium / IBC)
3. Prof. Dr. Enny Sudarmonowati (National Research and Innovation Agency/BRIN)
4. Prof. Dr. Ernawati Sinaga (Universitas Nasional of Indonesia / UNAS)
5. Dr. Ratih Asmana Ningrum (National Research and Innovation Agency/BRIN)
6. Prof. Dr. Ilya Raskin (Rutgers University, USA)

Organizing Committee:
Chair : Ida Ayu Agung Paramitaswari, S.Si. (Universitas Mahasaraswati / UNMAS, Denpasar-Bali)
Vice Chair :
1.Dr. Ir. Nonon Saribanon, M.Si. (Universitas Nasional/UNAS, Jakarta)
2.Dr. Saptowo J. Pardal, MS. (Indonesian Biotechnology Consortium / IBC, Bogor)
Treasurer :
1.Woro Umayi Ananda, S.Si., M.Si. (Indonesian Biotechnology Consortium/ IBC, Bogor)
2.Dr. I. Gusti Agung Sri Rwa Jayantini (Universitas Mahasaraswati/ UNMAS, Denpasar-Bali)
Website and IT : Yosafat Dedi Wijaya (Indonesian Biotechnology Consortium / IBC, Jakarta)
Logistics, Transportation and Accomodation
1.Ida Ayu Purnama Bestari, S.Pd., M.Sc (Universitas Pendidikan Ganesha / UNDIKSHA, Singaraja - Bali)
2.Apt. Maria Malida Vernandes Sasadara, S.Farm., MP (Universitas Mahasaraswati/ UNMAS, Denpasar-Bali)
Public Relation and Sponsorship:
1. Sara Wijayanti, S.Si (Indonesian Biotechnology Consortium / IBC, Jakarta)
2. Sigit Sadewo, S.Si., M.Si (Indonesian Biotechnology Consortium / IBC, Jakarta)
3. Fadilla Dewi Rakhmawaty (Indonesian Biotechnology Consortium / IBC, Jakarta)

Scientific Committee:
Chair : Dr.rer.nat Bernadetta Rina, M.Si. (National Research and Innovation Agency/BRIN, Bogor)
Vice Chair : Hayati Minarsi, M.Sc., Ph.D (Indonesian Biotechnology Consortium / IBC, Bogor)
Scientific Committee members:
1.Prof. Dr. Abu Amar (Indonesian Biotechnology Consortium / IBC, Jakarta)
2.Dr. Rossa Yunita, M.Si. (Indonesian Biotechnology Consortium / IBC, Bogor)
3.Dr. Irma Indrayani, SIP, M.Si. (Universitas Nasional/UNAS, Jakarta)
4.Dr. Ni Gst.Ag. Gde Eka Martiningsih (Universitas Mahasaraswati/ UNMAS, Denpasar-Bali)
5.Dr. Putu Eka Pasmidi Ariati (Universitas Mahasaraswati/ UNMAS, Denpasar-Bali)

Workshop :
1. Alvira Noer Effendy, S.Si, M.Si (Universitas Nasional/UNAS, Jakarta)
2. Hanifa Nur Adilah, S.Si, M.Si (Universitas Nasional/UNAS, Jakarta)
3. Fenti Widiastuti (Indonesian Biotechnology Consortium / IBC, Jakarta)
4. I Made Ardaka, M.Si (Eka Karya Botanic Garden, Bedugul-Bali)
5. Tuah Malem Bangun, M.Si. (Eka Karya Botanic Garden, Bedugul-Bali)

Chair of Organizing Committee
Ida Ayu Agung Paramitaswari

PAPER • **OPEN ACCESS**

Peer Review Statement

To cite this article: 2023 *IOP Conf. Ser.: Earth Environ. Sci.* **1255** 011002

View the article online for updates and enhancements.

You may also like

- Peer Review Statement
- Peer Review Statement
- Peer Review Statement

245th ECS Meeting • May 26-30, 2024 • San Francisco, CA

Learn more & submit!

Present your work at the leading electrochemistry & solid-state science conference.

Network with academic, government, and industry influencers!

Submit abstracts by December 1, 2023

This content was downloaded from IP address 182.253.116.215 on 24/11/2023 at 17:20

Peer Review Statement

All papers published in this volume have been reviewed through processes administered by the Editors. Reviews were conducted by expert referees to the professional and scientific standards expected of a proceedings journal published by IOP Publishing.

- **Type of peer review:** Double Anonymous
- **Conference submission management system:** Morressier
- **Number of submissions received:** 93
- **Number of submissions sent for review:** 93
- **Number of submissions accepted:** 82
- **Acceptance Rate (Submissions Accepted / Submissions Received × 100):** 88.2
- **Average number of reviews per paper:** 2.024390243902439
- **Total number of reviewers involved:** 22
- **Contact person for queries:**
  Name: Bernadetta Rina Hastilestari
  Email: bern003@brin.go.id
  Affiliation: Republik Indonesia - Badan Riset dan Inovasi Nasional Republik Indonesia

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd

IOPscience

PAPER • OPEN ACCESS

Tobacco resistance gene expression levels in response to the infection of *Phytophthora nicotianae*

J Sukweenadhi[1], C V Christian[1], J Setiabudi[1] and W D Savitri[1]

Published under licence by IOP Publishing Ltd

IOP Conference Series: Earth and Environmental Science, Volume 1255, The International Conference and Workshop in conjuction with the 8th Indonesia Biotechnology Conference (IBC) 16/05/2023 - 18/05/2023 Bali, Indonesia

Citation J Sukweenadhi *et al* 2023 *IOP Conf. Ser.: Earth Environ. Sci.* **1255** 012049

DOI 10.1088/1755-1315/1255/1/012049

sukwee@staff.ubaya.ac.id

[1] Faculty of Biotechnology, University of Surabaya, Raya Kalirungkut, Surabaya 60293, Republic of Indonesia

Buy this article in print

Journal RSS

Sign up for new issue notifications

Create citation alert

Abstract

One of the main obstacles often encountered in efforts to increase productivity and quality of tobacco (*Nicotiana tabacum*) yields is the attack of black shank disease by the oomycetes *Phytophthora nicotianae*. So far, what has been done mainly by tobacco cultivators is the observation of resistance through calculating the rate of death or disease of plants due to pathogen attack, so research is needed to determine the resistance profile of local varieties of tobacco to black shank disease in molecular stage to speed up the screening process of tobacco varieties. This study aim to determine the gene expression profile related to resistance in tobacco varieties Beinhart 1000, Hick Broadleaf, and two local varieties, namely Dark 302 and Dark 314, in response to black shank disease. The research consisted of preparing tobacco and *P. nicotianae* inoculum, artificial inoculation, followed by semi-quantitative analysis of gene expression related to tobacco resistance

This site uses cookies. By continuing to use this site you agree to our use of cookies. To find out more, see our Privacy and Cookies policy.

PR1, PR4, PLP2, and PUB24. Tobacco resistant variety, Beinhart 1000, expressed a relatively 285% higher PR1 significantly than the control. The length of time of infection showed that PR1, PR4, PLP2, and PUB24 genes were expressed temporally, and the dynamics of expression of each test gene affected the development of pathological conditions.

Export citation and abstract BibTeX RIS

Previous article in issue

Next article in issue

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.

You may also like

This site uses cookies. By continuing to use this site you agree to our use of cookies. To find out more, see our Privacy and Cookies policy.

This site uses cookies. By continuing to use this site you agree to our use of cookies. To find out more, see our Privacy and Cookies policy.

PAPER • **OPEN ACCESS**

Tobacco resistance gene expression levels in response to the infection of *Phytophthora nicotianae*

To cite this article: J Sukweenadhi *et al* 2023 *IOP Conf. Ser.: Earth Environ. Sci.* **1255** 012049

View the article online for updates and enhancements.

245th ECS Meeting • May 26-30, 2024 • San Francisco, CA

Learn more & submit!

Present your work at the leading electrochemistry & solid-state science conference.

Network with academic, government, and industry influencers!

Submit abstracts by December 1, 2023

This content was downloaded from IP address 114.125.125.196 on 24/11/2023 at 04:40

Tobacco resistance gene expression levels in response to the infection of *Phytophthora nicotianae*

J Sukweenadhi*, C V Christian, J Setiabudi, W D Savitri

Faculty of Biotechnology, University of Surabaya, Raya Kalirungkut, Surabaya 60293, Republic of Indonesia
*E-mail: sukwee@staff.ubaya.ac.id

Abstract. One of the main obstacles often encountered in efforts to increase productivity and quality of tobacco (*Nicotiana tabacum*) yields is the attack of black shank disease by the oomycetes *Phytophthora nicotianae*. So far, what has been done mainly by tobacco cultivators is the observation of resistance through calculating the rate of death or disease of plants due to pathogen attack, so research is needed to determine the resistance profile of local varieties of tobacco to black shank disease in molecular stage to speed up the screening process of tobacco varieties. This study aim to determine the gene expression profile related to resistance in tobacco varieties Beinhart 1000, Hick Broadleaf, and two local varieties, namely Dark 302 and Dark 314, in response to black shank disease. The research consisted of preparing tobacco and *P. nicotianae* inoculum, artificial inoculation, followed by semi-quantitative analysis of gene expression related to tobacco resistance, PR1, PR4, PLP2, and PUB24. Tobacco resistant variety, Beinhart 1000, expressed a relatively 285% higher PR1 significantly than the control. The length of time of infection showed that PR1, PR4, PLP2, and PUB24 genes were expressed temporally, and the dynamics of expression of each test gene affected the development of pathological conditions.

1. Introduction

"*Lanas*", also known as the black shank, caused by the oomycetes *Phytophthora nicotianae,* is a major disease of tobacco worldwide. *Phytophthora nicotianae* is a soil pathogen from the Stramenopiles-Alveolata-Rhizaria subgroup [1]. This pathogen can spread rapidly under conditions of 23-28C temperature and high soil moisture [2]. This disease is detrimental to farmers because it can attack tobacco plants at all ages, both as seedlings and adults, especially in the root, stem, and leaf organs [2–4]. Symptoms of infection that arise are wilting plants, the base of the stem being rotten and appearing brownish, and the appearance of insulation in the pith when splitting [5]. Research that has been done before showed the influence of local Indonesian tobacco varieties with lanas disease resistance, namely 4 out of 10 varieties (Dark A, Dark B, Jepon Pote, and Marakot) were determined to be more resistant to *P. nicotianae* attack [6]. Four tobacco varieties will be studied in this research, namely Beinhart-1000, Hick Broadleaf, Dark 302, and Dark 314. Beinhart-1000 is one of the tobacco varieties resistant to *P. nicotianae* infection, while Hick Broadleaf is an example of a susceptible variety [7]. The specific resistance of Dark 302 and Dark 314 tobacco varieties to *P. nicotianae has* not yet been studied.

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd

So far, what has been done by tobacco farmers in the field is the observation of resistance by calculating the level of death or illness of plants due to pathogen attacks. Therefore, it is necessary to research to determine molecularly the gene expression profile associated with tobacco resistance to malignant disease to accelerate the *screening of* tobacco varieties. The selected tobacco plant resistance-related genes are *Pathogenesis related 1* (*PR1*), *Pathogenesis related 4 PR4* [4], while susceptibility-related genes are *Patatin-like protein 2* (*PLP2*) and *Plant U-box 24* (*PUB24*) [8]. The purpose of this study is to determine whether there are differences in the level of gene expression in tobacco varieties Beinhart-1000, Hick Broadleaf, Dark 302, and Dark 314 in response to infection with *P. nicotianae*, as well as the effect of the length of time of infection with *P. nicotianae* on gene expression. The hypothesis formulated for this study is that there are differences in gene expression related to resistance to *Phytophthora nicotianae* infection in tobacco plants of Beinhart-1000, Hick Broadleaf, Dark 302, and Dark 314 varieties.

2. Materials and methods

2.1. Plants materials

Observations and preliminary research were carried out from August to December 2021, and the main research continued from January to July 2022. The research was conducted in the Laboratory of Biotechnology Microorganism, Laboratory of Purification and Molecular Biology, and greenhouse of the Laboratory of Plant Biotechnology, Faculty of Biotechnology, University of Surabaya. The independent variables studied in this study were tobacco plant varieties, namely Beinhart-1000, Hick Broadleaf, Dark 302, and Dark 314. The dependent variable observed was the relative expression of *PR1*, *PR4*, *PLP2*, and *PUB24* in the test tobacco, with the *L25* gene as the *housekeeping gene*. The control variables in this study were the amount of inoculant soil given as an infectious agent, the age of tobacco of each variety, the *sampling* time point, and the growth conditions of tobacco in the greenhouse.

2.2. Tobacco preparation

Tobacco seedlings were seeded by sprinkling 0.07 g of tobacco seeds evenly onto the wet soil surface. After 14 days after sowing (das), the seedlings were transferred to the nursery *tray* using tweezers. At 60 das, the tobacco seedlings were transferred to *polybags* containing 10 liters of growing media. The growing media used consisted of a mixture of compost fertilizer and media *mix* in a ratio of 5:1. The media *mix* contained river soil, *cocopeat*, and husk charcoal in a ratio of 4:3:2. Tobacco fertilization was done by applying 25 mL of KNO fertilizer solution$_3$ with a concentration of 1.5 g/L to the soil of each *polybag*. Tobacco seedlings planted in the *polybags* were maintained until 144 das before the next treatment was finally carried out. In this study, 48 tobacco plants were used, of which 24 were included in the treatment group while the rest were controlled.

2.3. Isolation of Phytophthora nicotianae culture from infected tobacco

Candidate cultures of *Phytophthora nicotianae* were isolated from the stems of tobacco plants experiencing symptoms of malignant disease in the field. The culture isolation method refers to the method conducted by [9]. The tobacco stems from being taken were first washed with running water, split, and surface sterilized in 0.5% sodium hypochlorite solution for 30 s, then rinsed using sterile distilled water. The inside of the dried stem was then placed in Corn Meal Agar (CMA) medium aseptically to be incubated for 1-2 days at room temperature (±25ºC). Subculturing was done three times to purify the candidate culture from contaminant microorganisms.

Macrogen carried out molecular identification of isolate species through the *Internal Transcribed Spacer* (ITS) *Region Sequencing* program. The ITS region of *ribosomal* DNA (rDNA) was amplified using a pair of common primers, namely ITS4 and ITS5. The primer sequences used were ITS5 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). The ITS1-5.8S-ITS2 sequences obtained were then analyzed using the BLAST *tool* on the NCBI *web server*.

2.4. Preparation of Phytophthora nicotianae starter inoculum

Rolled oat grains needed to grow cultures were sterilized by autoclaving at 121° C for 15 min. After that, 5 agar culture plugs were aseptically placed into a small jar containing 25 g of oat grains, and 10 mL of sterile distilled water was added. Five small culture jars of oat grains were incubated in the dark for 5 days at room temperature. Then the 5 small jars of oat grains culture were aseptically transferred to a larger jar containing 250 g of oat grains and 100 mL of sterile distilled water. Incubation was carried out for 20 days. A total of 365 g of oat grains culture was mixed with 1.926 g of soil taken from around the roots of tobacco infested with malaria in the field, forming an inoculum concentration of 18.95% w/w to be inoculated into the treated tobacco.

2.5. Artificial inoculation and tobacco leaf sampling

Artificial inoculation of tobacco was done by sprinkling and mixing the surface soil of tobacco growing media with 95 g of inoculum. Artificial inoculation was carried out in the afternoon in the rainy season when the air humidity was high (78.25±5)%, while the air temperature was low (26.2±1.3)° C, thus favoring disease infection by *Phytophthora nicotianae*. Young non-necrotic tobacco leaves from each variety, both control and treatment groups, were taken on days 0, 1, 2, 3, and 9 after artificial inoculation (dai = days after inoculation) of *P. nicotianae* inoculum. The leaves were picked, put into *aluminum foil* bags, and then liquid nitrogen was added to freeze the leaves.

2.6. RNA extraction and cDNA synthesis

According to the manual instructions, RNA extraction was performed using an RNA extraction kit (Favorgen, FavorPrepTM Plant Total RNA Mini Kit). Approximately 100 mg of tobacco leaf powder was placed in a 1.5 mL microtube for total RNA extraction. Complementary DNA (cDNA) chains were prepared from tobacco RNA extracted at the previous stage using reverse transcription. In this study, the SMOBIO Reverse Transcription Kit RP1400 was used according to the manual instructions. Tobacco cDNA solution is stored in a freezer at -20° C until the time to be used.

2.7. Gene expression analysis

PR1, *PR4*, *PLP2*, and *PUB24* expression were analyzed using the PCR method followed by electrophoresis. The composition of the mixture used was 1 µL of cDNA *template* that had been diluted twice, 0.5 µL of *forward* primer (10 µM), 0.5 µL of *reverse* primer (10 µM), 2 µL of SMOBIO ExcelTaq *master mix*™ 5x PCR Master Dye Mix or 5 µL of Abclonal PowerPol 2x PCR Mix RK20719 *master mix*, and sterile ddH_2O to reach a mixture volume of 10 µL. The temperature settings in each PCR reaction stage are pre-denaturation (94°C, 2 min); denaturation (94°C, 30 s); *annealing* (58.5°C for *PR1*, 62°C for *PR4*, 60.2°C for *PLP2*, 55.4°C for *PUB24*, 65.7°C for *L25*, 30 s); elongation (72°C, 30 s), and final elongation (72°C, 1 min). The cycle used for *PR1* amplification was 28 cycles, while *PR4*, *PLP2*, and *PUB24* were 36 cycles. Gene expression analysis results using PCR and electrophoresis methods produce data in black-and-white photos of amplicons from the gel observed under UV light. Calculating relative gene expression begins with analyzing the thickness or intensity of the amplicons using ImageJ *software* that has been calibrated to produce data in the form of peak areas. The thickness of the amplicons of the test genes, namely *PR1*, *PR4*, *PUB24*, and *PLP2*, was compared with the *housekeeping* gene, *L25*, to obtain the relative expression value of the gene.

2.8. Analysis of sequencing results

Sequence correction of sequencing results was carried out with the help of SeqMan Pro DNAStar software. Sequencing results from both primers, namely ITS4 and ITS5 primers, were entered, then combined through the "*Assemble"* menu and observed the combined results (*contigs*) that were successfully obtained. Contig sequences were identified using the BLAST Nucleotide (BLASTn) NCBI web server. A *Neighbor-Joining* phylogenetic tree showing the relationship between the isolated culture ITS region sequencing results and the ITS regions of *Lasiodiplodia pseutheobromae* and *Phytophthora nicotianae* was created using Molecular Evolutionary Genetics Analysis (MEGA) 11 software.

2.9. Statistical analysis

Statistical data analysis was carried out with the help of IBM SPSS Statistics 26 software. The data normality test used the Kolmogorov-Smirnov method, followed by the data homogeneity test, then the test for differences in gene relative expression was carried out using the *Mann-Whitney* method because the data was found to be not normally distributed, although homogeneous. Data is called significantly different when it has a P *value* <0.05.

3. Results

3.1. Black shank disease infection in tobacco

Root tobacco sections from the treatment groups, Beinhart-1000, Hick Broadleaf, Dark 302, and Dark 314 varieties, were inoculated with soil from the area around the roots of malignant tobacco in the field. Disease incidence was observed from the day of inoculation (0 dai), until 50 days later (50 dai). Based on observations, Hick Broadleaf tobacco experienced sudden death at 3 dai (experienced by 3 out of 6 plants), followed by dying at 9 dai. (Fig. 1A), and ended in death at 20 dai. Symptoms of stem rot and stem base rot were also experienced by Dark 302 (all plants) and Dark 314 (3 out of 6 plants) tobacco varieties, observed at 20 dai. "*Lanas*" symptoms were observed on all Dark 314 tobacco varieties at 30 hsi and died at 50 dai. The lower stems of Hick Broadleaf, Dark 302, and Dark 314 tobacco varieties were split to observe the formation of lesion borders (Fig. 1B). In contrast, until the observation at 50 dai, Beinhart-1000 tobacco appeared healthy and without disease symptoms, indicating its resistance to infection.

Figure 1. The appearance of malignant disease symptoms; Hick Broadleaf tobacco dying, experiencing malignant disease symptoms of chlorosis, stem rot, and decay at 9 dai (A); Necrotic schist and hyphae at the base of a longitudinally sectioned tobacco stem (B).

3.2. Expression Analysis of PR1, PR4, PLP2, and PUB24

A temporal comparison of the relative expression of *PR1*, *PR4*, *PLP2*, and *PUB24* can be observed in Figure 2. A significant increase in *PLP2* expression against the control group occurred in Dark 302 tobacco at 2 dai, which was 124% higher than the control. A significant increase in *PUB24* expression against the control group occurred in Hick Broadleaf tobacco at 3 dai, 83% greater than the control. In tobacco variety Beinhart-1000, there was a significant increase in *PR1* expression at 1 dai, reaching 285% higher than the control, where this phenomenon was not observed in the other 3 varieties. Interestingly, at 3 dai, the day when the first symptoms of senescence appeared in the susceptible variety, Hick Broadleaf, little *PR1* expression was observed, even the lowest among the other 3 varieties, which was 64% of the control. The significant increase in *PR1* at 1 hsi is a response to Beinhart-1000's resistance to malignant disease infection, where it is known that PR1 is a *downstream marker of* the defense response controlled by the salicylic acid signaling pathway [10].

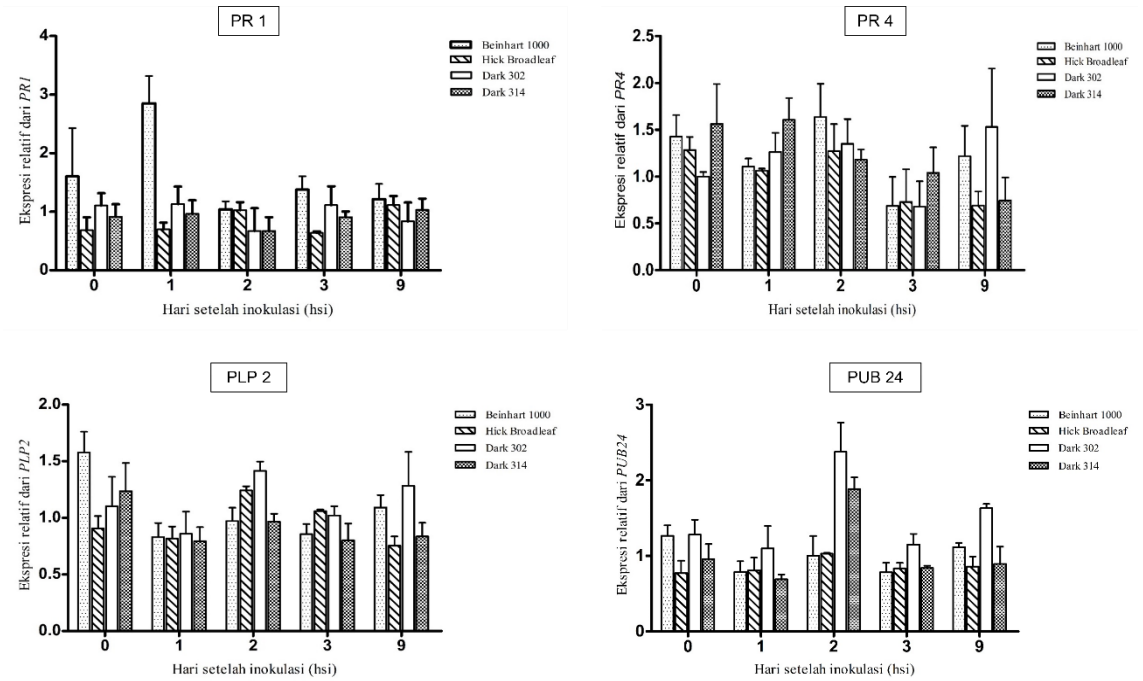


Figure 2. Relative expression graph of *PR1*, *PR4*, *PLP2*, and *PUB24* genes from Beinhart-1000, Hick Broadleaf, Dark 302, and Dark 314 tobacco varieties. Error bars show the standard deviation of 3 replicates. Expression levels are normalized to the housekeeping gene (*L25*) and relative expression of the control group.

Notes: *P < 0.05: significantly different from control.

A significant increase in *PR4* expression against the control group occurred in Dark 314 tobacco varieties at 1 dai, which increased by 161% compared to the control. In field practice, Dark 314 is a medium-resistant variety, so this significant increase in *PR4* data indicates a different defense strategy between Beinhart-1000 and Dark 314. The increase in *PR4* is related to the role of PR4 as a *downstream marker of* the defense response mediated by the jasmonic acid and ethylene signaling pathways [10].

3.3. Isolate culture sequencing results

Analysis of the sequencing results performed by Macrogen shows the sequence similarity between the isolated culture and *Lasiodiplodia pseutheobromae,* which has a sequence similarity identity of 99%, *e-value* 0.0, and *coverage* 96%. The results of sequence improvement and phylogenetic tree analysis also show the same conclusion, namely, the isolated culture has a close relationship with the species *Lasiodiplodia pseutheobromae*, so it is identified as *Lasiodiplodia pseutheobromae*. So far, there have been no reports of *L. pseutheobromae* infection in tobacco. It is suspected that the dominant pathogenic agent in the soil mixture and *infested oats* that could attack the test tobacco was *P. nicotianae* spores, while *L. pseutheobromae,* an endophytic organism that can grow in plant tissues without causing disease symptoms, was isolated when taking samples from diseased tobacco stems.

4. Discussion

4.1. Persistence of Phytophthora nicotianae in soil and environmental factors for successful P. nicotianae infection

Phytophthora nicotianae inoculated on the test tobacco in this study need to invade *host* cells to obtain nutrients and fulfill its life cycle, so it has strategies and attack weapons that support it in infecting susceptible tobacco varieties effectively. On the other hand, in response to biotic stress from phytopathogenic microorganisms, tobacco develops a self-defense system that aims to limit the movement and resist the growth of pathogens, namely in the form of pathogen-associated molecular patterns-triggered immunity (PAM-TI/PTI), and effector-triggered immunity (ETI). The interaction

dynamics of the tobacco immune response against pathogen attack involves the expression of various phytohormones and proteins that support tobacco resistance and susceptibility. In this study, it was shown that differences in the expression profiles of several resistance genes (*PR1*, *PR4*) and susceptibility (*PLP2*, *PUB24*) related genes among tobacco varieties temporally early in the infection period might affect the success of tobacco against *P. nicotianae* infection.

Soil from tobacco growing sites infected with "*Lanas"* can be used as an infectious agent due to the presence of thick-walled spores of *Phytophthora* spp., which can survive in the soil for a long time[11], 6 years for chlamydospores and 4 years for oospores, even after the tobacco tissue has decomposed. Hyphae and sporangia, and later zoospores, can develop naturally from oospores and chlamydospores when the soil is moist. When suitable host plants are present, either sporangia, hyphae or zoospores can infect the host, thus restarting the disease cycle. The inoculum of *P. nicotianae* oospores and chlamydospores that persist in the soil for a long time often become the main and primary inoculum that causes malaria in the field [8]. It seems that the strategy of using spore survival is successfully applied in this study; soil inoculation can cause symptoms of malignant disease in tobacco because spores survive. Appropriate environmental factors are also required for successful pathogen infection, where *P. nicotianae* infection has been successfully carried out on 30-40 days old tobacco in an environment with a temperature of 22-26°C and relative humidity of 65-70%. [1]. The results of thermometer and hygrometer measurements in the afternoon during the sampling period showed that the greenhouse where the test tobacco was infected had an average temperature of 26.2^{o} C and a relative humidity of 78.25%, thus supporting the infection of *P. nicotianae* into the roots of the test tobacco.

The successful invasion of *P. nicotianae is* characterized by its ability to invade the active cambium in the stem and colonize nearby transport tissues [12]. Lack of nutrients and water caused the test tobacco variety Hick Broadleaf leaves to wilt at 3 dai. At 9 dai, it was observed that the continuous inhibition of nutrient distribution caused stem tissue to die, characterized by dryness at the base of the stem and discoloration from green to brown, as well as severe leaf chlorosis (Fig. 1A). Further invasion of *P. nicotianae* results in the death of all tobacco plant tissues, the formation of insulation in the dead stem vessel network, as well as root rot. This progression of malignant disease infection was also observed in Dark 314 and Dark 302 varieties but at a longer period than Hick Broadleaf. This indicates the role of varietal differences in the defense response to *P. nicotianae* infection.

4.2. PR1 role and expression in P. nicotianae-treated-tobacco plant defense

PR1 plays an important role in tobacco defense against *P. nicotianae* because it can act as a sterol binder important in the growth and development of *P. nicotianae* as a sterol auxotroph organism. PR1 can bind sterols in the plasma membrane of *P. nicotianae* through the C-terminal domain of the cysteine-rich secretory protein (CAP), causing the death of the pathogen [13]. PR1 is a small size of 4 kDa protein. In addition to being secreted outside the cell to interact with *P. nicotianae*, high concentrations of PR1 directly were also found to accumulate in the cell vacuole as a more effective advanced defense that is launched when the pathogen successfully penetrates the plant cell wall [14]. Overexpression of PR1 protein has been widely reported to increase plant resistance to *P. nicotianae* by binding sterols [15], but the molecular mechanism to obtain this resistance is not yet known.

In this study, PR1 gene expression in the Beinhart-1000 variety increased significantly at 1 dai, whereas this event was not observed in the other 4 varieties, either at 1 hsi or all *sampling* points. The achievement of peak PR1 gene expression in the first 24 hours after inoculation is in line with the results of Ali *et al*. (2018) on *Altenaria brassicae* infection in *Brassica juncea* and Li-Xia *et al*. (2012) on *Plasmopara viticola* infection in *Vitis vinifera* [16,17]. While Bao *et al*. (2019) observed that *PR1* expression in resistant tobacco variety TKF2022 inoculated with *P. nicotianae* continued to increase from 0 hours after inoculation (hai), 12 hai, 24 hai (1 dai), and reached peak expression at 72 hai [4]. High *PR1* expression generally indicates the Systemic Acquired Resistance (SAR) response by Beinhart-1000 tobacco varieties to suppress the growth of the biotrophic phase of *P. nicotianae*, in addition to sterol binding activity by PR1 [18].

Systemic Acquired Resistance can protect uninfected tissues, so the distribution of SA to leaves conveys signals for expressing defense-related genes, such as *PR1*. It seems that this increase in *PR1* at the beginning of *P. nicotianae* infection has successfully suppressed the growth of the pathogen so that the formation of *P. nicotianae* colonies is not maximized; the infection of tobacco fails. Another supporting research proved that the growth of *Phytophthora brassicae was* inhibited by 90% by treatment with PR1 protein [19]. The opposite condition occurs in Dark 302, Dark 314, and susceptible varieties, namely Hick Broadleaf, which express little *PR1*, making it easy to be resisted by *P. nicotianae* colonies [20]. Other results are supported by the research of Bao *et al*. (2019) where PR1 gene expression by susceptible tobacco variety TKF4321 was observed to increase dramatically at 96 hai or 4 dai [4]. This is thought to be due to differences in genotypes between tobacco varieties affecting the ability of tobacco to respond to SA signals into *PR1* transcription.

4.3. PR4 role and expression in P. nicotianae-treated-tobacco plant defense

The N-terminal hevein-like (CB-HEL) domain of PR4 can bind to chitin on the cell wall of pathogens thereby supporting *host* resistance to pathogens such as fungi [21]. The cell wall of the *oomycetes* P. *nicotianae is* not composed of chitin, but cellulose (β-1,4-glucan), β-1,3 and β-1,6-glucans [22]. However, previous studies have shown that overexpression of PR4 protein confers resistance to *P. nicotianae* [23]. This is supported by another activity of PR4 besides chitinase, which is the degradation of pathogenic RNA (ribonuclease; RNAse) by the C-terminal hevein-like *(*CD-HEL) domain of PR4. The presence of amino acid residues His11 and His110 on the active side of the CD-HEL domain of Arabidopsis PR4 provides ribonuclease activity that strengthens *host* resistance to fungal pathogens, namely through the mechanism of cutting phosphodiester bonds according to the acid-base reaction of enzymes such as RNAse A [21,24].

In this study, a significant increase in *PR4* expression occurred against the control by Dark 314 tobacco varieties at 1 dai, and was observed to be maintained higher than the control until 3 dai. This suggests that the activity of the tobacco defense response through the JA and ET pathways affects the time course of malignant disease development, which counteracts the development of *P. nicotianae* so that symptoms only appear at 20 dai. Susceptible varieties, namely Hick Broadleaf, do not express much *PR1* and *PR4*, two types of PR protein groups that can support plant resistance, so pathogen growth is poorly controlled. Increased expression of *PR4* in the resistant medium tobacco, Dark 302, was observed from 9 his. This result is similar to the results of Bao *et al*. (2019) that after being inoculated with *P. nicotianae, the* resistant tobacco variety, TKF2002, expressed more *PR4* than the susceptible variety, and maintained its high expression until 3 dai [4]. Another study by Bai *et al*. (2013) showed that *PR4* transcription in *Malus domestica* (*MdPR4*) increased by 8.58 times from the control in response to *B. dothidea* inoculation at 30 dai [25].

Many researchers have described the antagonistic relationship between SA and JA pathway plant defense signaling, as well as the synergism of JA and ET. JA signaling can reduce SA accumulation by activating the transcription factor NAC, thus inhibiting the expression of *isochorismate synthase 1* (ICS1), which is a SA biosynthesis gene [26]. This can explain the event that occurred in this study, when *PR1 was* highly expressed at 1 hsi by Beinhart-1000 tobacco, but decreased dramatically the next day, replaced by highly expressed *PR4* at 2 dai, representing the inhibition of SA accumulation. Although JA/ET defense pathways, including *PR4* expression, generally play a role against necrotrophic organisms, previous studies have found its expression responds to SA induction, also against hemibiotrophic organisms such as *P. nicotianae* early in its life cycle because it is expressed higher than the control starting 6 h after inoculation [4,25]. Increased expression of *PR1* and *PR4* in tobacco infected with *P. nicotianae* has also been known to be in line with increased production of $H\ O_{22}$ which acts as a detoxification response to increased levels of *reactive oxygen species* (ROS) in the PTI response [4].

4.4. PLP2 role and expression in P. nicotianae-treated-tobacco plant defense

Patatin-like protein *2* (PLP2) has a *catalytic dyad* consisting of serine and aspartic acid amino acid residues on its active side to perform its function as a *lipid acyl hydrolase* (LAH). In response to

pathogens, *NtPLP2* can act as a modulator gene that promotes cell death, and is also involved in oxylipin metabolism starting from the hydrolysis of chloroplast membrane galactolipids and phospholipids. Silencing of PLP2 gene expression is known to increase *Arabidopsis* resistance to the necrotrophic organism *B. cinerea* and the hemibiotrophic *P. syringae* while *wild type Arabidopsis* experiences more severe disease symptoms [27]. In contrast, hydrolysis of phospholipids or galactolipids can produce fatty acids as precursors of the defense reaction jasmonic acid, thereby increasing the resistance of Arabidopsis to *cucumber mosaic virus* (CMV) infection. The process of cell death in plant defense mechanisms is tightly controlled and balanced to maximize pathogen growth restriction through hypersensitive reactions, but also inducing defenses against pathogens. PLP2 activity can increase in the presence of necrosis stimuli so that more cell death occurs, which is when the balance shifts in favor of the hypersensitive reaction (HR).

High expression of *PLP2* by Dark 302 and Hick Broadleaf tobacco at 2 hsi may play a role in tobacco susceptibility to *P. nicotianae* infection; in line with the previous report [8,27]. When associated with the possibility ki nan hydrolase results to be a precursor in the biosynthesis of jasmonic acid, the event can be observed at 9 hsi where the expression pattern of *PLP2* coincides with *PR4* which is a *marker* gene of the JA signaling pathway. Tobacco variety Dark 302 expressed the highest amount of *PLP2* in relative terms, and *PR4 was* also observed; while Hick Broadleaf expressed the least. This indicates that high JA activity is supported by an adequate supply of its precursors from hydrolysis by PLP2 protein. Phospholipids or galactolipids can be hydrolyzed and produce products in the form of fatty acids (*polyunsaturated fatty acid*; PUFA), then catalyzed by lipoxygenase (LOX) enzyme activity to produce FA-hydroperoxide, then metabolized into JA [28].

4.5. PUB4 role and expression in P. nicotianae-treated-tobacco plant defense

The plant u-box E3 ubiquitin ligase protein (PUB24) plays an important role in the ubiquitin-proteosome pathway, which can determine the specificity of protein targets to be degraded through its substrate-recognizing domain. In the context of plant defense, ubiquitin ligases can play a role in regulating the concentration of defense-related proteins so that they only accumulate when the plant is under stress, then eliminate them when they are no longer needed, for example in regulating the elimination of the phosphorylated WRKY70 transcription factor (WRKY70-P) when there is no pathogen attack [29]. However, several studies have shown that the ligase function of E3 PUB24 can also play a negative role in the plant defense system against pathogens, including *P. nicotianae*.

The increased expression of *E3 PUB24* in the test tobacco indicates the influence of susceptibility-related genes during the development of disease infection. The results of this study are in line with the results of Meng *et al*. (2021) as well as Trujillo *et al*. (2008), which concluded that the relative expression of *E3 PUB24* in tobacco variety XHJ increased from 6 hai, reached peak expression at 12 hai, and was kept high until 60 hai played a role in increasing plant susceptibility by suppressing the PTI response [8,30], Mutations in *PUB24* (*pub24*) from Arabidopsis inoculated with the *H. parasitica* caused an extension of the duration and accumulation of ROS due to increased expression of the gene encoding the *ROS* production enzyme, *RbohD*, as well as an increase in *marker* genes for PTI, namely *OX1*, *WRKY29* [30]. On the other hand, *oomycetes* can secrete effectors to suppress the PTI response of plants, such as Avr3a protein from *P. infestans*. This synergism of pathogenic effectors and negative effects of E3 PUB24 explains the relationship between highly expressed *E3 PUB24* in test tobacco and tobacco susceptibility. The time at which the *host* defense response is activated plays a role in determining *host* susceptibility and resistance because *host* susceptibility can occur not only due to the *host's* inability to express resistance-associated proteins, but also too late to inhibit the growth of pathogens have already colonized. Differences in gene expression dynamics between tobacco varieties in this study have provided a molecular basis for the resistance of the resistant variety Beinhart-1000 and the susceptible variety Hick Broadleaf to *P. nicotianae*, as well as a comparison with the local varieties Dark 302 and Dark 314. In this study, 6 test tobacco varieties of Beinhart-1000 were observed to be healthy until the 50th day after inoculation (50 dai). Beinhart-1000 tobacco actively expressed resistance-related genes, namely *PR1* starting at 1 hsi and *PR4* starting at 2 dai.

4.6. Genetic distance analysis and disease symptoms in P. nicotianae-treated-tobacco

Genetic distance analysis showed that the average kinship score between the land isolate culture and *P. nicotianae* was 0.7344 while with *L. pseutheobromae* was 0.0051. The smaller score means that the kinship between the isolate culture and *L. pseutheobromae* is closer, that is, there are fewer sequence differences, in this case there are at least 5 different bases in each 1000 bases analyzed. However, the disease symptoms caused in the test tobacco in this study did not match the disease symptoms caused by *Lasiodiplodia pseutheobromae,* namely *dieback* on the trunk and branches, splitting of the bark and wedge-shaped rot on woody trees [31], as well as gummosis, thus supporting the statement that *L. pseutheobromae* may be an endophytic fungus that was picked up during culture isolation.

5. Conclusion

Throughout infection, gene expression dynamics continue to occur and affect tobacco defense in addition to *P. nicotianae* resistance. *PR1*, *PR4*, *PLP2*, and *PUB24* are just 4 of the many genes involved in tobacco defense. The resistant tobacco variety, Beinhart-1000, expressed *PR1* at a relative level of 285%, significantly higher than the control. The mechanism of tobacco resistance and susceptibility given from the results of this study is a theoretical conjecture, so it needs to be further investigated to the proteomic level. The length of time of infection shows that genes are temporally expressed and the dynamics of expression of each test gene affect the development of pathological conditions. To further this research, focus on proteomic analysis and identification of additional resistance genes while exploring gene expression dynamics, environmental factors, and epigenetics. Develop molecular markers for resistance breeding to efficiently develop new tobacco cultivars with improved resistance to black shank disease.

Acknowledgments

We would like to express our gratitude to the Faculty of Biotechnology, University of Surabaya, for their invaluable support throughout this research project. Additionally, we would like to extend our thanks to Billy, an alumnus of Biotechnology, for generously providing all of the necessary research materials.

References

[1] Vanegas-Villa D M, Navarro-Álzate R A, Afanador-Kafuri L, Gutiérrez-Monsalve J A, Morales-Osorio J G, Uribe-Soto S I and Gaviria-Gutiérrez B M 2020 *Nematology* **22** 1179–92
[2] Guo D, Yuan C, Luo Y, Chen Y H, Lu M, Chen G, Ren G, Cui C, Zhang J and An D 2020 *Pestic. Biochem. Physiol.* **165** 104523
[3] Gallup C A, Sullivan M J and Shew H D 2006 *The Plant Health Instructor* DOI: 10.1094/PHI-I-2006-0717-01
[4] Bao Y, Ding N, Qin Q, Wu X, Martinez N, Miller R, Zaitlin D, Li D and Yang S 2019 *Molecular Breeding* **39** 1–10
[5] Steede W T, Ma J M, Eickholt D P, Drake-Stowe K E, Kernodle S P, Shew H D, Danehower D A and Lewis R S 2017 *Plant Dis.* **101** 1214–21
[6] Suhara C, Hidayah N, Penelitian B, Pemanis T, Serat D, Karangploso J R and Pos K 2020 *Buletin Tanaman Tembakau, Serat dan Minyak Industri* **12** 22–33
[7] Van Jaarsveld E, Wingfield M J and Drenth A 2002 *J. Phytopathology* **150** 456–62
[8] Meng Y, Zhang Q, Ding W and Shan W 2014 *Mycology* **5** 43–51
[9] Henuk J B D, Sinaga M S and Hidayat S H 2017 *Biodiversitas* **18** 1100–8
[10] Attard A, Gourgues M, Callemeyn-Torre N and Keller H 2010 *New Phytol.* **187** 449–60
[11] Hwu F Y, Lai M W and Liou R F 2017 *Front Microbiol* **8**

[12] Dodd R S, Hüberli D, Mayer W, Harnik T Y, Afzal-Rafii Z and Garbelotto M 2008 *New Phytol.***179** 505–14
[13] Kazan K and Gardiner D M 2017 *PLoS Pathog.* **13** (5) e1006297
[14] Schneiter R and Di Pietro A 2013 *Biomol. Concepts* **4** 519–25
[15] Sarowar S, Young J K, Eui N K, Ki D K, Byung K H, Islam R and Jeong S S 2005 *Plant Cell Rep.* **24** 216–24
[16] Ali S, Mir Z A, Bhat J A, Tyagi A, Chandrashekar N, Yadav P, Rawat S, Sultana M and Grover A 2018 *Biotech* **8** 1–14
[17] Li-Xia H, Chao G, Yong-Mei C, Fang-Gui Z and Xin L 2012 *Plant Physiol.* **48** 57–62
[18] Mazumder M, Das S, Saha U, Chatterjee M, Bannerjee K and Basu D 2013 *Plant Physiol. Biochem.* **70** 43–51
[19] Gamir J, Darwiche R, van't Hof P, Choudhary V, Stumpe M, Schneiter R and Mauch F 2017 *Plant Journal* **89** 502–9
[20] Breen S, Williams S J, Outram M, Kobe B and Solomon P S 2017 *Trends Plant Sci.* **22** 871–9
[21] Bertini L, Proietti S, Aleandri M P, Mondello F, Sandini S, Caporale C and Caruso C 2012 *Chemistry* **393** 1533–46
[22] Judelson H S and Blanco F A 2005 *Nat. Rev. Microbiol.* **3** 47–58
[23] Naidoo R, Ferreira L, Berger D K, Myburg A A and Naidoo S 2013 *Front Plant Sci.* **4** 43
[24] Caporale C, Di Berardino I, Leonardi L, Bertini L, Cascone A, Buonocore V and Caruso C 2004 *FEBS Lett.* **575** 71–6
[25] Bai S, Dong C, Li B and Dai H 2013 *Plant Physiol. Biochem.* **62** 23–32
[26] Verma V, Ravindran P and Kumar P P 2016 *BMC Plant Biol.* **16**
[27] La Camera S, Geoffroy P, Samaha H, Ndiaye A, Rahim G, Legrand M and Heitz T 2005 *Plant J.* **44** 810–25
[28] La Camera S, Balagué C, Göbel C, Geoffroy P, Legrand M, Feussner I, Roby D and Heitz T 2009 *Mol. Plant-Microbe Interact.* **22** 469–81
[29] Liu H, Liu B, Lou S, Bi H, Tang H, Tong S, Song Y, Chen N, Zhang H, Jiang Y and Liu J 2021 *New Phytol.* **230** 1095–109
[30] Trujillo M, Ichimura K, Casais C and Shirasu K 2008 *Curr. Biol.* **18** 1396–401
[31] Dong W 2020 *Asian J. Mycol.ph* **3** 399–407

Source details

IOP Conference Series: Earth and Environmental Science

Scopus coverage years: from 2010 to Present
ISSN: 1755-1307 E-ISSN: 1755-1315

Subject area: Earth and Planetary Sciences: General Earth and Planetary Sciences
Environmental Science: General Environmental Science

Source type: Conference Proceeding

View all documents > Set document alert Save to source list Source Homepage

CiteScore 2022
0.8

SJR 2022
0.197

SNIP 2022
0.255

CiteScore CiteScore rank & trend Scopus content coverage

Improved CiteScore methodology
CiteScore 2022 counts the citations received in 2019-2022 to articles, reviews, conference papers, book chapters and data papers published in 2019-2022, and divides this by the number of publications published in 2019-2022. Learn more >

CiteScore 2022

0.8 = 61,816 Citations 2019 - 2022 / 75,404 Documents 2019 - 2022

Calculated on 05 May, 2023

CiteScoreTracker 2023

0.9 = 59,377 Citations to date / 62,879 Documents to date

Last updated on 08 November, 2023 • Updated monthly

CiteScore rank 2022

Category	Rank	Percentile
Earth and Planetary Sciences — General Earth and Planetary Sciences	#150/192	22nd
Environmental Science — General Environmental	#182/227	20th

View CiteScore methodology > CiteScore FAQ > Add CiteScore to your site

About Scopus

What is Scopus

Content coverage

Scopus blog

Scopus API

Privacy matters

Language

日本語版を表示する

查看简体中文版本

查看繁體中文版本

Просмотр версии на русском языке

Customer Service

Help

Tutorials

Contact us

ELSEVIER

Terms and conditions ↗ Privacy policy ↗

All content on this site: Copyright © 2023 Elsevier B.V. ↗, its licensors, and contributors. All rights are reserved, including those for text and data mining, AI training, and similar technologies. For all open access content, the Creative Commons licensing terms apply.
We use cookies to help provide and enhance our service and tailor content. By continuing, you agree to the use of cookies ↗.

RELX™