BIOVALENTIA: Biological Research Journal
Vol. 10 No. 2 (2024)
DOI: 10.24233/biov.10.2.2024.236
ISSN Online: 2477-1392
Pages: 140-144

Identification of Castor Genotypes Resistant to Graymold in Peatland Area based on Resistance Gene Analogs: A Preliminary Study

Ninik Nihayatul Wahibah[1*], Atria Martina[1], Arini[1], Yasir Sidik[2]
[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, University of Riau, Kampus Bina Widya Km 12.5 Simpang Baru 28293 Pekanbaru, Riau, Indonesia*
[2]*Department of Biology, Faculty of Education, University of Muhammadiyah Surakarta*

[*]Corresponding author
E-mail address: ninik.nihayatul@lecturer.unri.ac.id

Abstrak: *Graymold is one of devastating fungal disease of castor plants (Ricinus communis L.) caused by Botryotinia ricini. Resistant germplasm is the basic and essential sources to support castor breeding obtaining disease resistant varieties. Objective of this study was to identify castor genotypes resistant to gray mold in peatland agroforestry area in Kepulauan Meranti that naturally infected by graymold fungal pathogen. This is the first report about castor plants cultivated under peat soil. Infected and uninfected castor plants were sampled and analyzed their Resistance Gene Analog (RGA). Results showed that resistant plants expressed diverse phenotype and amplified DNA fragment using RGA primers. The fragments were similar to ABC transporter gene which is one of RGA classes. Additionally, the fragments revealed P-loop NTPase conserved domain. In contrast, all infected plants failed to produced PCR product. The results indicated that uninfected castor plants can be used as source of resistant trait. Their RGA sequences can be analyzed to develop molecular marker for supporting gray mold resistant breeding program.*
Keywords: *castor, graymold, peatland, resistance, RGA*

Received:11 November 2021 , Accepted: 27 November 2024, Publish: 27 November 2024

1. INTRODUCTION

Castor plants (*Ricinus communis* L.) is one of most important non-edible oil seeds that can be used in a various industries such as cosmetics, medicine and biofuel. mold Castor plants produced non-edible oil important for a wide range of industries such as cosmetics, medicine and biofuel [1]. Castor-based agroforestry on peat land area in Kepulauan Meranti has inevitably been attacked by gray mold disease. This is the first castor field grown under peat soil area. Based on our previous study, this agroforestry consist of several different genotypes
based on morphological traits [2] and molecular markers (RAPD and SRAP; data not shown). Under natural infected area, uninfected genotypes are potential germplasms provide resistant sources.

Gray mold has been reported as the most devastating disease and difficult to deal with, particularly compared to two other major diseases in castor plants, vascular wilt and charcoal rot [3]. Gray mold disease results in significant reduction of castor seeds productivity [4]. The causal agent of gray mold disease is fungal pathogen *Botryotinia ricini* or *Amphobotrys ricini* at anamorph stage [5]. *B. ricini* is categorized as seedborne disease with the main target is inflorescence inflorescence [6]. Early symptoms can be recognized by bluish spots on growing racemes [7]. This symptom can easily be spread particularly in windy or rainy condi-

tion leading to fruit sterility and damage other castor plant organs.

Resistant variety is the best approach to deal with disease problem in various crops. Resistance Gene Analogs (RGAs) are candidate

didate *R* genes that can contribute to assess resistant plant genotypes. Identification of resistant genotype is a pre-requisite step in resistant breeding program. For these reasons, objective of this study was to identify castor genotype resistant to graymold and analyze RGA sequence.

2. MATERIALS AND METHODS

Plant material and DNA isolation

Infected and uninfected plant materials were sampled in castor agroforestry located in Desa Tanjung Peranap, Pulau Tebing Tinggi Riau. Gray mold fungal infection naturally happened in the field. Infected fruits were collected for further fungal identification in the laboratory and plant morphological characters were observed. Genomic DNA of infected and uninfected plants were extracted from leaves tissue using Genomic DNA Mini Kit (Plant) (Geneaid, Taiwan). Method of DNA isolation followed manufacturer's protocol.

Amplification and sequencing

DNA amplification used Thermo Scientific DreamTaq DNA (Thermo Fisher Scientific Inc., USA). Primers sequence flanking the RGA region (Table 1) referred to Kapadia et al. (2015). total of 50 µL PCR mixture consisted of 5 µL of 10x DreamTaq Buffer, 5 µL of 2 mM dNTP, 5 µL of 10 µM Forward and Reverse primers, and 2.5 U DNA Taq Polymerase. PCR cycle consisted of 5 min at 95^{o}C, 30 sec at 94 ^{o}C, 30 sec at different annealing temperature, 1 min at 72 ^{o}C, and 10 min at 72 ^{o}C. PCR products were visualized using 1% gel agarose under UV filtered camera. DNA ladder of 1 kb was used to estimate size of the amplified fragments. PCR products then were sent to PT. Genetika Science Indonesia for sequencing at 1st Base-Asia Malaysia.

Table 1: *Primers used in this study based on* [8]

Primer*	Primer Sequence (5'-3)	Domain *R* Gene
P.3 (F)	GGACCTGGTGGGGTTGGGAA ;ACAAC	P-loop, RPS2
P.4 (R)	CAACGCTAGTGGCAATCC	GLPL, RPS2
P.9 (F)	GGTGGGGTTGGGAAGACAAC G	P-loop, RPS2, L5
P.10 (R)	CCACGCTAGTGGCAATCC	GLPL, RPS2, L6
P.11 (F)	GGAATGGGNGGNGTNGGNAA RAC	P-loop, RPS2
P.12 (R)	YCTAGTTGTRAYDATDAYYY TRC	RNBS-B, RPS2

*F= forward; R- reverse

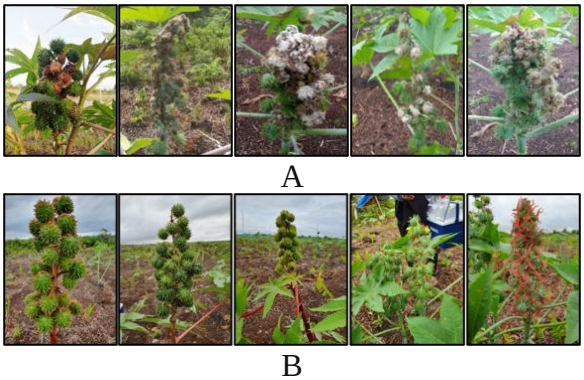
Data analysis

Phenotype data were described qualitatively. DNA sequence data were checked by BioEdit and aligned to database at NCBI https://www.ncbi.nlm.nih.gov/ and TAIR https://www.arabidopsis.org/. To assess specific domain, DNA sequence were subjected to Consereved Domain Database at NCBI (CDD NCBI) https://www.ncbi.nlm.nih.gov/ Structure/cdd.

Sampling site of this study is located at 102^{o} 29' 20.147" E; 0^{o} 51' 54.745" N under peat soil type with pH 4.76-4.79. This is the first castor agroforestry cultivated under peat soil type. We sampled infected and uninfected castor within same area under natural-infection of gray mold fungal disease. Symptom of gray mold disease on infected plants is easily observed in the field. It can be recognized by grayish dust colored raceme. It hass been reported that the main target of the fungal infection is raceme (inflorescence and capsule) which can be at any developmental crop stage. The fungus prefer to infect inflorescence, particularly pistillate flowers, primarily due to their high water content. We sampled infected castor raceme for further isolation. Our microscopic assessment (data not shown) confirmed the isolates as gray mold fungus, *Bortyotinia ricini*.

Sampled plants expressed varied phenotype (Figure 1). Morphological variation were observed on raceme, stem, and leave such as size of prickle on fruit surface (long and short prickles), stem color (red, mahogany, green), wax layer on stem (waxy, without wax), internode (elongated and densed) and leaf shape (flat, concave). Pigmentation on castor stem has been considered as expressed product of secondary metabolites such as anthocyanin. These metabolites contribute to particular roles in plant resistance against fungal pathogen [9]. Accumulation of anthocyanin also enhanced resistance against gray mold in tomato plants [10]. Other study investigated that castor genotype with presence of prickle and condensed internode also showed wilt resistance trait [11].

Genetic variation amongst castor genotypes in this study will facilitate castor breeding program for selecting and improving the genetic traits particularly uninfected plants. Uninfected castor genotypes survived in the sampling site are potential as germplasm source for resistance against gray mold. Selection for disease resistant genotypes under field observation can be the first step in supporting resistance breeding program. Therefore we further analyze molecular characteristic related to resistant trait by amplification using RGA primers.



A

B

Figure 1: *Infected and uninfected castor plants grown in peatland agroforestry in Kepulauan Meranti Riau Indonesia*

Genomic DNA was successfully extracted from ten samples (five infected and five uninfected plants). Three of five primer pairs produced clear bands. Only uninfected genotypes amplified DNA fragments using all three primer pairs (Figure 2). Primer pair P3+P4, B and C annealed to four, three and two uninfected plants, respectively and generated DNA fragment around 600-1000 bp. In contrast, all infected plants did not amplify DNA template for any primer pair. The results indicated that infected plants does not have DNA region that complement to primer sequence. It means that DNA segment which is detected using RGA primer is different compared to uninfected plants that amplified DNA bands. The amplified DNA fragments are likely related to *R* gene controlling disease resistant trait.

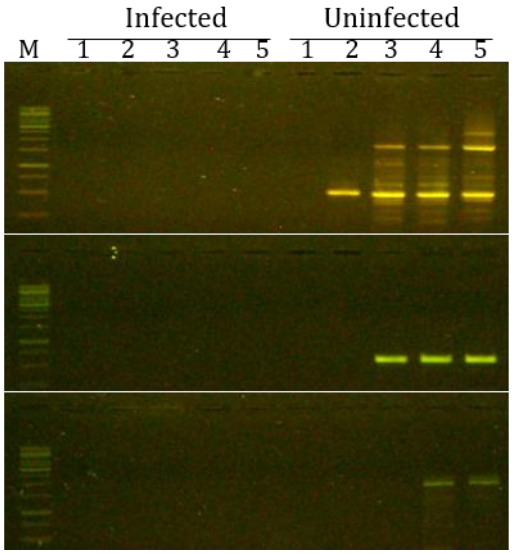


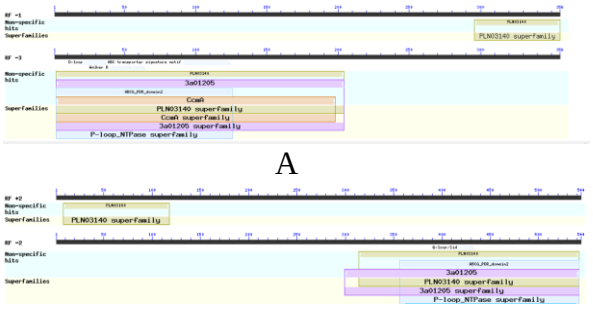
Figure 2: *Result of DNA amplification using three primer pairs, P.1+P.4, P.9+P.10, and P.11+P.12, from top to bottom.*

To confirm that RGA region were covered within PCR product, we further analyze sequence of the amplicons. Two fragments amplified using primers P.3 + P.4 and P.9 + P.10 are about 500 bp. Similarity assess-

ment based on NCBI showed that the fragments are resemble to predicted *Ricinus communis* ABC (ATP-binding cassette) transporter G family member 36 or ABCG36 gene (accession no. XM-002515924) with e-value of 4e-65. Additionally, based on TAIR database, our fragments show significant similarity to sequence encoding AtABCG36 protein (accession no. AT1G59870.1) with e-value of 1e-24.

ABC transporter is categorized as one of RGA classes in plants [12]. The proteins primarily function in translocation a lot of substrates across plasma membrane powered by ATP molecules. Plasma membrane-localized ABCG36 protein is also called PENETRATION (PEN) 3 or PLEIOTROPIC DRUG RESISTANCE8 (PDR8) involved in plant-pathogen defense. AtABCG36/PDR8/PEN3 protein involve in PAMP-triggered immunity (PTI) signaling pathway, defense respons to fungus, regulation of defense response by callose deposition, response to chitin, and response to molecule of fungal origin. Pattern recognition receptors (PRRs) bind to pathogen-associated molecular patterns (PAMPs) leading to PTI. It has been known as the first layer of inducible defense response in plants. While the second layer is known as effector-triggered immunity (ETI) involving *R* gene products. RGAs constitute both PRRs and *R* genes.

ABC transporter protein cover highly conserved region including the presence of a phosphate-binding loop (P-loop or Walker A motif) [13]. To confirmed the conserved domain, we analyze conserved domain using CDD NCBI database. The results confirmed that both fragments constitute P-loop(phosphate binding loop) NTPase superfamily (Figure 3).



A

B

Figure 3: Fragments amplified by P.3 + P.4 (A) and P.9 + P.10 (B) revealed P-loop NTPase conserved domain based on CDD NCBI

4. CONCLUSION

Our study found infected and uninfected plants in the same area naturally infected by graymold disease. Their phenotypes show genetic variation. Uninfected plants amplified RGA fragments that is ABC transporter with P-loop NTPase conserved domain. In contrast, infected plants failed to generate PCR product. It can be concluded that RGA potential to be applied as marker for graymold disease resistance in castor plants. Further studies will be required to more gain insight into the RGA structure and mechanism related to graymold disease resistance in castor plants.

5. ACKNOWLEDGEMENTS

We appreciate DIPA LPPM Universitas Riau Skema Penelitian Bidang Ilmu with Project Number 688/UN19.5.1.3/ PT.01.03/2021 for the financial support and Bapak Benny Jatmiko, ST (PT. Meranti Energy Alam) for facilitating our field work.

REFERENCES

[1] B. Z. Salihu, A. K. Gana, and B. O. Apuyor, "Castor Oil Plant (Ricinus communis L .): Botany , Ecology and Uses," *Int. J. Sci. Res.*, vol. 3, no. 5, pp. 1333–1341, 2014.

[2] N. N. Wahibah, V. J. Yahya, Fitmawati, M. Agung, and R. Budiono, "Morphological Variation of Castor Bean (*Ricinus communis* L .) on Peatland Area in Kepulauan Meranti Riau Indonesia," *J. Phys.: Conf. Ser.* 1655 012028, 2020.

[3] A. Yeboah, "Botryotinia ricini (Gray Mold); A Major Disease in Castor Bean (*Ricinus communis* L.)," *Int. J. Pure Appl. Biosci.*, vol. 7, no. 4, pp. 8–22, 2019.

[4] L. S. Severino *et al.*, "A review on the challenges for increased production of castor," *Agron. J.*, vol. 104, no. 4, pp. 853–880, 2012.

[5] D. Jos, "Gray Mold of Castor: A Review," *Plant Pathol.*, no. October, 2012.

[6] D. K. Sharma and S. Rana, "Seed-Borne and post-harvest disease of Castor bean (*Ricinus communis* LINN) and their management : A Review," *J. Phytol. Res.*, vol. 30, no. 1, pp. 31–45, 2017.

[7] E. Sayed, H. Ziedan, A. G. Attallah, S. K. Abd-el-aal, and A. F. Sahab, "Molecular Identification And Pathogenic Potential of Botrytis Cinerea Isolates Causing Fruit Blight Of Cucumber Under Protective Greenhouse in Egypt," vol. 18, no. 2, pp. 1563–1569, 2018.

[8] C. V. Kapadia, M. K. Mahatma, M. J. Parekh, P. Nafisa, and R. S. Tomar, "Identification of resistance gene analogs (RGAS) from highly wilt resistant castor (Ricinus communis L.) genotype," *Res. J. Biotechnol.*, vol. 10, no. 5, pp. 16–26, 2015.

[9] A. R. Da Silva *et al.*, "Characterization and performance of castor bean lineages and parents at the UFRB germplasm bank," *PLoS One*, vol. 14, no. 1, pp. 1–15, 2019.

[10] Y. Zhang *et al.*, "Anthocyanins double the shelf life of tomatoes by delaying overripening and reducing susceptibility to gray mold," *Curr. Biol.*, vol. 23, no. 12, pp. 1094–1100, 2013.

[11] J. Lu *et al.*, "RcPAL, a key gene in lignin biosynthesis in Ricinus communis L.," *BMC Plant Biol.*, vol. 19, no. 1, pp. 1–11, 2019.

[12] M. K. Sekhwal, P. Li, I. Lam, X. Wang, S. Cloutier, and F. M. You, "Disease resistance gene analogs (RGAs) in plants," *Int. J. Mol. Sci.*, vol. 16, no. 8, pp. 19248–19290, 2015.

[13] S. Wilkens, "Structure and mechanism of ABC transporters," *F1000Prime Rep.*, vol. 7, no. February, pp. 1–9, 2015.