

Oil Palm Tolerant to Basal Stem Rot Using Genome-Wide Association Studies (GWAS) Method

ABSTRACT

Basal stem rot caused by *Ganoderma boninense* is a serious threat to oil palm (*Elaeis guineensis*) plantations, resulting in significant economic losses. The transmission of *G. boninense* in parent plants occurs through contact between roots and sources of inoculum. Dikaryotic mycelia from *G. boninense* form needle-like structures that help penetrate host cells. Subsequently, basidiocarps form at the base of infected oil palm trees, causing basal stem rot disease. Therefore, it is necessary to develop varieties that are tolerant to *G. boninense*. GWAS is used to identify associations between genetic variations (usually single nucleotide polymorphisms, or SNPs) and specific traits across the genome. The application of Genome-Wide Association Studies (GWAS) is an alternative method to identify genetic markers associated with tolerance to basal stem rot in oil palms. ~~GWAS is used to identify associations between genetic variations (usually single nucleotide polymorphisms, or SNPs) and specific traits across the genome.~~ By utilizing GWAS, breeders can accelerate the development of oil palm varieties resistant to this disease.

1. INTRODUCTION

Palm oil is one of the key plantation commodities playing an important role in global oil production due to its ability to produce crude palm oil and palm kernel oil, essential raw materials for both food and non-food industries [1]. The oil palm plant has numerous benefits for human life [2]. Practical uses of this oil include cooking oil and margarine, which are staple products widely used by various groups [3]. Palm oil is not only utilized as a source of healthy cooking oil but also as a raw material for various industries, such as biodiesel, cosmetics, pharmaceuticals, chemicals, textiles, paper, bioenergy, and putty [4]. Given the many benefits of palm oil, its demand continues to increase. To meet this demand, efforts to boost production are necessary. However, cultivation activities aimed at increasing production often face numerous challenges.

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One major issue in oil palm cultivation is pest and disease attacks. Pests and diseases affecting oil palm include leaf rot disease (*Phytophthora palmivora*), bunch rot disease (*Marasmiuspalmivorus*), stem rot disease (*Thielaviopsis paradoxa*), crown shedding disease (*Fusarium oxysporum*), anthracnose disease (*Colletotrichum gloeosporioides*), red rust disease (*Rigidoporusmicroporus*), moko disease (*Ralstonia solanacearum*), and basal stem rot disease (*Ganoderma boninense*) [5][6][7].

Basal stem rot, caused by *G.boninense*, is the most destructive disease. Symptoms in affected plants include yellowing of old leaves, necrosis, rot at the base of the stem, and eventually plant death [8]. This disease attacks the lower stem, leading to reduced production and plant mortality, posing a serious threat to the sustainability of palm oil production [9]. Controlling this disease can be done by using disease-resistant seedlings, maintaining plantation sanitation, crop rotation, and applying fungicides [10].

To develop oil palm varieties tolerant to *Ganoderma*, a combination of several technologies is often used. Steps that can be taken include identifying resistance genes using molecular markers, GWAS, or genomic technologies to pinpoint resistance genes. Subsequent selection and breeding utilize these resistance genes or markers in breeding programs. The breeding lines are then propagated through tissue culture techniques. Finally, field trials are conducted to test the new varieties in the field to ensure their resistance and productivity.

Comment [PM2]: Provide losses due to basal stem rot

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2. BASAL STEM ROT DISEASE

Basal stem rot is a common symptom of the disease caused by *G.boninense* in oil palm plants. In Indonesia, this disease can significantly reduce palm oil productivity per hectare, primarily due to plant mortality, which can exceed 50%, resulting in economic losses of up to 43% [11] [12].

G. boninense, belonging to the *Ganodermataceae* family and *Basidiomycetes* class, is a white-rot fungus capable of degrading the lignin component of wood. It is the most virulent *Ganoderma* species causing severe basal stem rot [13]. Other *Ganoderma spp.* that can cause basal stem rot include *G. applanatum*, *G. boninense*, *G. chaliceum*, *G. lucidum*, *G. miniatocinctum*, *G. pseudoferreum*, and *G. tomatum*. However, the primary species causing the disease is *G. boninense*[14][15].

The transmission of *G. boninense* occurs through contact between roots and sources of inoculum, such as stumps, stems, and diseased palm [16]. Monokaryotic mycelia are not pathogenic to oil palm trees, while pathogenic mycelia are dikaryotic [17]. Dikaryotic mycelia, through hyphal morphogenesis, create needle-like structures that help penetrate host cells. Subsequently, basidiocarps form at the base of infected trees, leading to infection by *G. boninense*[18][19].

3. BREEDING BASAL STEM ROT TOLERANT PLANTS

One way to combat *G. boninense* attacks is by using plants resistant to *G. boninense*. To date, there is no vertical resistance to basal stem rot [20]. However, there is horizontal and partial resistance, which does not prevent infection but reduces or compensates for damage [21].

Breeding programs have utilized *E. oleifera* and *E. guineensis* species as genetic resources due to their different resistance levels [22]. In recent decades, basal stem rot-tolerant oil palm progenies have been obtained from crosses such as Cameroon x Cameroon, Cameroon x AVROS, and Nigeria x Nigeria [23]. Resistance genes and spatial resistance to *G. boninense* were derived from crosses like Zaire x Cameroon and Congo x Cameroon [24]. Partial resistance sources were also found in *E. oleifera* and *E. guineensis* lines from Malaysia and Indonesia [25].

Potential genetic sources for resistance have been identified in La Mé A x Deli Dabou B and Yangambi IRHO A x Deli Socindo [26]. Another source of spatial resistance is 'Yangambi Ganoderma-Tolerant 1,' released by FELDA Global Ventures [27].

Non-conventional breeding methods have been initiated, such as increasing lignin content. Lignin protects the structure and function of oil palm cell walls, playing a crucial role in plant defense. Efforts to increase lignin content are pursued through in vitro breeding. However, in vitro breeding is a lengthy process requiring optimal transformation methods [28].

4. OIL PALM BREEDING USING GENOME-WIDE ASSOCIATION STUDIES (GWAS)

The development of disease-resistant oil palm varieties is crucial for sustainable production. Traditional breeding methods are slow and often ineffective due to the polygenic nature of disease resistance. Modern genomic tools, such as Genome-Wide Association Studies (GWAS), offer a promising solution for identifying genetic markers associated with disease resistance, enabling more efficient breeding strategies.

GWAS is a method used to identify associations between genetic variations (typically single nucleotide polymorphisms, or SNPs) and specific traits across the genome [29]. Disease-resistant oil palm breeding programs have been developed by evaluating the genotypes and phenotypes of germplasm as genetic material sources. Using GWAS can detect extensive genetic variations among individuals in a population. The analysis of associations between single nucleotide polymorphisms (SNPs) can determine important traits such as disease resistance.

GWAS for producing disease-resistant plant varieties has been applied to several commodities, including Fusarium wilt resistance in cotton, white mold (*Sclerotinia sclerotiorum*) resistance in beans [30], and powdery mildew and rust resistance in

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barley[31]. The use of GWAS for oil palm resistance to basal stem rot (BSR) is still limited. The used GWAS to detect chitinase genes in oil palm EgCHI1, EgCHI2, and EgCHI3. Chitinase enzymes (poly[1,4-N-acetyl- β -D-glucosaminidase, E.C. 3.2.1.14) hydrolyze chitin polymers to release N-acetyl glucosamine oligomers[32]. Detection of genes with structural defense properties, such as strengthening lignocellulosic components, and biochemical defenses, such as pathogenesis-related protein (PR-protein) genes that degrade pathogen cell walls, can prevent pathogen infection and colonization in plant tissues. *G. boninense* cell wall components in oil palm, consisting of chitin, glucan, and ergosterol, have been identified as PAMPs [12].

Comment [PM5]: Specify which gene

Genomic-based breeding in oil palm involves selecting individuals in a population based on phenotypic and genotypic characteristics. Using the GWAS method, genes with superior traits resistant to basal stem rot can be obtained [33]. The next steps include selection through limited-scale testing, high-throughput phenotyping, and seed technology digitization to increase oil palm productivity.

In summary, the scope of GWAS involves three main stages: phenotyping, genotyping, and linkage disequilibrium (LD) analysis[29]. Accurate association requires precise and accurate phenotyping methods and data collection. The collected data should have sufficient repetitions and, if possible, be conducted in different multi-location and multi-time conditions. Therefore, to avoid large errors, experiments must be managed with good research design methods to minimize environmental heterogeneity in the experiment.

Genotyping involves determining genetic markers, typically SNPs, used in GWAS. The goal of GWAS is to identify SNPs statistically associated with phenotypic traits. GWAS surveys thousands or even millions of SNPs, making it independent of linkage maps usually formed from bi-parental populations [34].

Linkage disequilibrium (LD) analysis in GWAS is due to recombination events occurring as individual species undergo mutations over time. This recombination between genes and markers in a population can provide information on the gene locations. Therefore, the more LD between markers and genes associated with target traits, the more precise and accurate the gene association[35].

The advantages of using GWAS in breeding programs include high-resolution mapping of genetic variations [36]. Detailed mapping of identified genome regions can uncover candidate genes related to disease resistance. Functional characterization of these genes will elucidate their roles in disease tolerance and facilitate the development of resistant varieties.

Recent GWAS studies have identified several key SNPs associated with basal stem rot tolerance [37]. These markers are located in genome regions linked to disease resistance pathways, such as defense response genes, pathogen recognition receptors, and signal transduction components.

GWAS also facilitates marker-assisted selection (MAS) in breeding and collaboration with other scientific fields. Collaborative research efforts and data sharing between researchers, breeders, and institutions can enhance the effectiveness of GWAS in oil palm breeding. Establishing comprehensive databases of phenotypic and genotypic data will facilitate the discovery of new genetic markers and their application in breeding programs.

Comment [PM6]: How GWAS inter-related with the MAS

5. CONCLUSION

Stem base rot disease caused by the fungus *Ganoderma Spp.* The transmission of *G. boninense* occurs through contact between roots and sources of inoculum, such as stumps, stems, and diseased oil palm trees. Monokaryotic mycelia are not pathogenic to oil palms, while pathogenic mycelia are dikaryotic. Dikaryotic mycelia form needle-like structures that help penetrate host cells. Subsequently, basidiocarps form at the base of infected oil palm trees. Therefore, it is necessary to develop varieties that are resistant to *Ganoderma Spp.* GWAS has emerged as a powerful tool for identifying genetic markers associated with basal stem rot tolerance in oil palm. By utilizing GWAS, breeders can accelerate the development of resistant varieties, ensuring sustainable palm oil production. Despite challenges, integrating GWAS with other genomic approaches and collaborative efforts will pave the way for significant advancements in oil palm breeding.

REFERENCES

1. Reinecke J, Von Hagen O, Manning S. Palm oil under discussion: the pros and cons of an agricultural raw material in sustainable food production. *Mod. Ernährung Heute Off. J. Food Chem. Inst. Assoc. Ger. Confect. Ind*; 2011.
2. Giri S, Bhatia S. Review on nutritional value and health benefits of palm oil. *Research & Review: Drugs and DrugsDevelopment*, 2020;2(1).
3. Aini IN, Miskandar MS. Utilization of palm oil and palm products in shortenings and margarines. *European Journal of Lipid Science and Technology*, 2007;109(4):422-432.
4. Rupilius W, Ahmad S. Palm oil and palm kernel oil as raw materials for basic oleochemicals and biodiesel. *European Journal of Lipid Science and Technology*, 2007;109(4):422-432.
5. Afandi D, Basyuni M, Putri L, Chalil D, Wati R, Siregar E, Syahputra I. Molecular performances of oil palm *Elaeis guineensis* tolerance to *Ganoderma sp.* *J. Phys. Conf. Ser.*, 2018;1116:052001.
6. Avila-Mendez K, Rodrigo Á, Araque L, Romero HM. Simultaneous transcriptome analysis of oil palm clones and *Phytophthora palmivora* reveals oil palm defense strategies. *PLoS ONE*, 2019;14:e0222774.

7. García-Gaona M, Botero-Rozo D, Araque L, Romero HM. The dynamic interaction between oil palm and *Phytophthora palmivora* in bud rot disease: Insights from transcriptomic analysis and network modelling. *J. Fungi*, 2024; 10(3):164.
8. Zakaria L. Basal stem rot of oil palm: the pathogen, disease incidence, and control methods. *Plant Disease*, 2023;107:603-615
9. Jazuli NA, Kamu A, Chong KP, Gabda D, Hassan A, Seman IA, Ho CM. A Review of Factors Affecting *Ganoderma* Basal Stem Rot Disease Progress in Oil Palm, *Plants*, 2022;11:2462.
10. Bivi MSHR, Paiko AS, Khairulmazmi A, Akhtar MS, Idris AS. Control of basal stem rot disease in oil palm by supplementation of calcium, copper, and salicylic acid. *Plant Pathol. J.* 2016; 32(5):396-406
11. Priwiratama H, Prasetyo AE, Susanto A. Incidence of basal stem rot disease of oil palm in converted planting areas and control treatments. *IOP Conf. Series: Earth and Environmental Science*, 2020;468:012036
12. Khoo YW, Chong KP. *Ganoderma boninense*: general characteristics of pathogenicity and methods of control. *Frontiers in Plant Science*, 2023;14:1156869.
13. Paterson RRM. *Ganoderma* disease of oil palm - a white rot perspective necessary for integrated control. *Crop Prot.* 2007; 26:1369–1376.
14. Turner PD. *Oil palm diseases and disorders*. New York: Oxford University Press; 1981.
15. Idris AS. Basal stem rot (BSR) of oil palm (*Elaeis guineensis* Jacq.) in Malaysia: factors associated with variation in disease severity. London: University of London; 1999.
16. Ariffin D, Idris AS, Singh G. Status of *Ganoderma* in oil palm. Eds. J. Flood, P. D. Bridge and M. Holderness. Wallingford, UK: CABI Publishing; 2000:49–68.
17. Freihofer D, Fowler TJ, Bartholomew K, Raudaskoski M, Horton JS, Kothe E. The mating-type genes of the basidiomycetes. Ed. J. Wendland. Cham, Switzerland: Springer; 2016:329–349.
18. Rees RW, Flood J, Hasan Y, Potter U, Cooper RM. Basal stem rot of oil palm (*Elaeis guineensis*), mode of root infection and lower stem invasion by *Ganoderma boninense*. *Plant Pathol*, 2009;8:982–989.
19. Govender N, Wong MY, Paterson R. Opportunities for new generation *Ganoderma boninense* biotechnology. Ed. H. Nevalainen. Cham, Switzerland: Springer; 2020:477–500.
20. Rozlihan FS, Jualang AG, Chong KP. Fatty acids and phenols involved in resistance of oil palm to *Ganoderma boninense*. *Adv. Environ. Biol.*, 2015;9: 11–16

21. WeningS, Rahmadi HY, Arif M, Supena N, Siregar HA, Prasetyo AE, Setiawati R D, Yenni Y, Suprianto E, Ernayunita, Faizah R, Sujadi, Susanto A PurbaA R. Construction of Ganoderma resistant oil palm planting material. in 6th IOPRI-MPOB International Seminar: Current research and management of pests, Ganoderma, and pollination in oil palm for higher productivity, Medan, North Sumatera Indonesia, Indonesia (Medan, Indonesia: Indonesian Oil Palm Research Institute). 2016: 1–14
22. Soh A. C. Breeding and genetics of the oil palm. Eds. O.M. Lai,C. P.Tan, C. C. Akoh. Urbana. IL AOCS Press; 2017:31–58.
23. Ariffin D, Idris AS, Kushairi A, Watt TA, Swinburne TR. Screening of oil palm for resistance to Ganoderma. Colloquium on Advances in Oil Palm Research, Bangi, Malaysia. 1999;12–17.
24. Idris AS, Ismail S, Ariffin D, Ahmad H. Prolonging the productive life of Ganoderma infected palms with hexaconazole. MPOB Inf. Ser. MPOB TT, 2000;214.
25. Durand-Gasselin T, Asmady H, Flori A, Jacquemard JC, Hayun Z., Breton, F, de Franqueville H. Possible sources of genetic resistance in oil palm (*Elaeisguineensis*Jacq.) to basal stem rot caused by *Ganoderma boninense* - prospects for future breeding. Mycopathol2005;159, 93–100
26. Turnbull N, de Franqueville H, Breton F, Jeyen S, Syahoutra I, Cochard B, Poulain V, Durand-Gasselin T. Breeding methodology to select oil palm planting material partially resistant to *Ganoderma boninense*. in 5th quadrennial international oil palm conference, Bali Nusa Dua Convention Center, Bali, Indonesia,2014:17
27. Tan JS, Lee YP, Alwee SLY, Kulandaivelu SSR, Mercière M, Zaremski A, Breton F. Fungal diseases affecting oil palm FELDA global ventures research and development, Malaysia. Ed. L. Camus Cambridge, UK: Burleigh Dodds Science Publishing; 2018.
28. Sahebi M, Hanafi MM, Wong MY, Idris AS, Azizi P, Jahromi MF,Shokryazdan P,Abiri R, Mohidin H. Towards immunity of oil palm against *Ganoderma fungus* infection. Acta Physiol. Plant., 2015; 37;1–16.
29. Uffelmann E, Huang QQ, Munung NS, de Vries J,Okada Y, Martin AR, Martin HC, Lappalainen T, Posthuma D. Genome-wide association studies. Nature Reviews Methods Primers, 2021;1:59
30. Campa A, García-Fernández C, Ferreira JJ. Genome-Wide Association Study (GWAS) for Resistance to *Sclerotinia sclerotiorum* in Common Bean. Genes, 2020;11, 1496.
31. Czembor JH, Czembor E, Suchecki R, Watson-Haigh NS.. Genome-Wide Association Study for Powdery Mildew and Rusts Adult Plant Resistance in European Spring Barley from Polish Gene Bank. Agronomy, 2022;12:7.
32. Aditama R, Tryono R, Tanjung, Utomo Z.A, Liwang T. Genome-wide identification of oil palm (*Elaeisguineensis* Jacq.) chitinases and their

response to *Ganoderma boninense* Pat. Infection. IOP Conf. Ser.: Earth Environ. Sci. 2019;293:012033.

33. Martin JJJ,Yarra R, Cao LWH. Oil Palm Breeding in the Modern Era: Challenges and Opportunities. Plants.2022;11:1395.
34. Adam Y, SamtalC, Brandenburg J, Falola O, Adebiji E. Performing post-genome-wide association study analysis: overview, challenges and recommendations.F1000Research 2021;10:1002.
35. Christoforou A, Dondrup M, Mattingsdal M,Mattheisen M, Giddaluru S, Nothen MM, Rietschel M, CichonS,Djurovic S, Andreassen OA, Jonassen I,Steen VM, Puntervoll PI,Hellard SL. Linkage-Disequilibrium-Based Binning Affects the Interpretation of GWASs. The American Journal of Human Genetics. 90:727–733.
36. Yabe S, Iwata H. Genomics-assisted breeding in minor and pseudo-cereals. Breeding Science, 2020;70:19–31.
37. Rahimi Y, Khahani B, Jamali A, Alipour H, Bihamta MR,Ingvarsson PK. Genome-wide association study to identify genomic loci associated with early vigor in bread wheat under simulated water deficit complemented with quantitative trait loci meta-analysis. Plant Genetics and Genomics G3, 2023; 13(2):jkac320.