

**IDENTIFICATION OF CITRUS RELATIVE, *MURAYA* SPP.
AND THE GENE EXPRESSION ANALYSIS ON CITRUS AND
MURAYA AGAINST SERRALYSIN TOXIN OF *CANDIDATUS*
LIBERIBACTER ASIATICUS**

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in Biotechnology



Submitted by

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**IDENTIFICATION OF CITRUS RELATIVE, MURAYA SPP.
AND THE GENE EXPRESSION ANALYSIS ON BOTH
CITRUS AND MURAYA AGAINST SERRALYSIN TOXIN OF
CANDIDATUS LIBERIBACTER ASIATICUS THE
HUANGLONGBING PATHOGEN**

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Yogyakarta, July, 2025

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Huanglongbing (HLB), yang disebabkan oleh *Candidatus Liberibacter asiaticus* (CLas), menjadi kendala utama produksi jeruk di Indonesia. Penelitian ini bertujuan mendeteksi infeksi CLas pada *Murraya* spp., menganalisis variasi genomiknya melalui Whole Genome Sequencing (WGS), serta mengevaluasi peran *serralysin-like* dalam respons tanaman terhadap infeksi. Sebanyak 13 aksesi dari tiga spesies *Murraya* (*M. paniculata*, *M. sumatrana*, *M. lucida*) dan satu hibrida dugaan (*M. omphalocarpa*) diidentifikasi secara morfologi dan molekuler. Infeksi CLas dikonfirmasi melalui PCR dan qPCR selama 14 bulan, dengan infeksi alami terdeteksi pada *M. paniculata* dan *M. sumatrana* di Pulau Jawa. Ini merupakan laporan pertama untuk *M. sumatrana*. Analisis WGS mengungkap variasi genetik dalam *Murraya* spp. Genom plastid *M. sumatrana*, *M. lucida*, dan *M. omphalocarpa* memiliki struktur kuadripartit dengan ukuran 158.427–160.165 bp. *M. sumatrana* membentuk klad terpisah dari *M. paniculata*, sementara sampel *M. lucida* dan *M. omphalocarpa* (Taiwan) kemungkinan merupakan spesies yang sama. Sampel *M. omphalocarpa* (350) diidentifikasi sebagai spesies baru, *M. cyclospensis*. Gen *ycf1* dan *ndhF* diusulkan sebagai penanda molekuler potensial untuk klasifikasi *Murraya*. Analisis *serralysin-like* menunjukkan perbedaan strategi pertahanan antara *Citrus* dan *Murraya* spp. Pada *Citrus* spp., *serralysin-like* kemungkinan memperparah penyakit bukan melalui konsentrasinya, melainkan dengan memicu produksi ROS berlebih yang mengaktifkan jalur asam salisilat (SA), menekan sinyal jasmonat (JA) dan etilen, serta menghambat fotosintesis, sehingga mempercepat kerusakan tanaman. Sebaliknya, *M. sumatrana* dan *M. paniculata* menunjukkan ketahanan melalui ekspresi Rubisco yang stabil, tingkat ekspresi *PR-15* yang tinggi, serta regulasi ROS yang lebih seimbang melalui interaksi *PR-15* dan *RbohD*. Meskipun mampu menjadi inang CLas, *Murraya* spp. tetap sehat, menunjukkan respon imun yang lebih efisien dan kerusakan oksidatif yang minimal.

Kata kunci: Huanglongbing, *Candidatus Liberibacter asiaticus* (CLas), *Murraya* spp., Whole Genome Sequencing (WGS), *serralysin-like*, PCR, qPCR, AUDPC, ROS, asam jasmonat, etilen, asam salisilat, rubisco.

Huanglongbing (HLB), caused by *Candidatus Liberibacter asiaticus* (CLas), is a major constraint on citrus production in Indonesia. This study aimed to detect CLas infection in *Muraya* spp., analyze genomic variation through Whole Genome Sequencing (WGS), and evaluate the role of serralysin-like in plant responses to infection. Thirteen accessions from three *Muraya* species (*M. paniculata*, *M. sumatrana*, *M. lucida*) and one putative hybrid (*M. omphalocarpa*) were morphologically and molecularly identified. CLas infection was confirmed through PCR and qPCR over 14 months, with natural infection detected in *M. paniculata* and *M. sumatrana* in Java. This is the first report of natural CLas infection in *M. sumatrana*. WGS analysis revealed genetic variation in *Muraya* spp. The plastid genomes of *M. sumatrana*, *M. lucida*, and *M. omphalocarpa* exhibited quadripartite structures, ranging from 158,427 to 160,165 bp. *M. sumatrana* formed a distinct clade from *M. paniculata*, while *M. lucida* and *M. omphalocarpa* (Taiwan) were likely the same species. Sample 350 of *M. omphalocarpa* was identified as a new species, *M. cycloensis*. The *ycf1* and *ndhF* genes were proposed as potential molecular markers for *Muraya* classification. Analysis of the *serralysin-like* gene revealed distinct defense strategies between *Citrus* and *Muraya* species. In *Citrus* spp., *serralysin-like* may worsen disease symptoms not by its concentration, but by inducing excessive reactive oxygen species (ROS) production. This triggers the salicylic acid (SA) pathway, suppresses jasmonic acid (JA) and ethylene signaling, and inhibits photosynthesis, thereby accelerating plant damage. In contrast, *M. sumatrana* and *M. paniculata* exhibit greater resilience through stable Rubisco expression, high *PR-15* levels, and balanced ROS regulation mediated by the interaction between *PR-15* and *RbohD*. Although they can host CLas, *Muraya* species remain healthy, indicating a more efficient immune response and reduced oxidative damage.

Keywords: Huanglongbing, *Candidatus Liberibacter asiaticus* (CLas), *Muraya* spp., Whole Genome Sequencing (WGS), serralysin-like, PCR, qPCR, AUDPC, ROS, jasmonic acid, ethylene, salicylic acid, rubisco.

I. INTRODUCTION

1.1. The Research Problem

Oranges are the world's most important fruit commodity, with an annual production exceeding 120 million tons. The most cultivated varieties include oranges (60%), mandarins (20%), and other citrus species such as tangerines, lemons, and kaffir limes (Scordino and Sabatino, 2014). In Indonesia, citrus cultivation is dominated by tangerines (70%), followed by mandarins (20%) and other citrus species (10%) (BPS, 2024). However, Indonesia's citrus productivity remains low, averaging 6.8 tons/ha/year, significantly lower than countries with advanced agro-industries, where yields can reach 20–40 tons/ha/year (Supriyatno et al., 2010; Moss et al., 2014).

A key factor limiting citrus production is Huanglongbing (HLB), also known as citrus greening, previously referred to as Citrus Vein Phloem Degeneration (CVPD) in Indonesia (Tirtawidjaja, 1964; Aubert, 1993; Texiera et al., 2005; Canales et al., 2016; Dala-Paula et al., 2019). HLB is caused by *Candidatus Liberibacter*, a phloem-restricted bacterium in the Proteobacteria phylum (Garnier et al., 1984; Jagoueix et al., 1994). Three species have been identified: *Ca. L. africanus* (CLaf), *Ca. L. asiaticus* (CLas), and *Ca. L. americanus* (CLam), based on 16S rRNA analysis (Bové, 2006). CLas and CLam are primarily transmitted by the Asian citrus psyllid (ACP: *Diaphorina citri*), while CLaf is transmitted by the African citrus psyllid (*Trioza erytreae*) (Bové, 2006; Reynaud et al., 2022). In Asia, including Java, CLas is the predominant pathogen, and *D. citri* is the primary vector (Aubert, 1987; Leong et al., 2022).