RESPONS FISIOLOGIS DAN DETEKSI GEN KETAHANAN TERHADAP MERKURI PADA *Aquarius palifolius* (Ness & Mart.) Christenh. & Byng

Mutiara Wahyu Nirmalasari

20/458309/BI/10542

Dosen Pembimbing : Dwi Umi Siswanti, S.Si.,M.Sc.

INTISARI

Tidak hanya industri berskala besar, tambang emas rakyat skala kecil menjadi penyumbang signifikan limbah logam berat dalam bentuk merkuri. Limbah merkuri yang tidak dikelola dengan baik akan menimbulkan berbagai permasalahan lingkungan dan kesehatan. Maka dari itu, diperlukan upaya bioremediasi untuk menurunkan nilai toksisitas merkuri di lingkungan. Bioremediasi dengan agen tanaman *Aquarius palifolius* dinilai berhasil mengurangi nilai toksisitas merkuri dengan mekanisme sintesis fitokelatin. Kemampuan *A. palifolius* sebagai tanaman hiperakumulator dapat ditinjau dari respons fisiologis pertumbuhannya (jumlah daun, tinggi dan diameter tangkai daun, pengamatan jaringan anatomi serta pengujian gen ketahanan yang meregulasi sintesis fitokelatin. Penelitian ini bertujuan untuk menganalisis respons fisiologis pertumbuhan, letak merkuri pada jaringan serta mendeteksi gen ketahanan *A. palifolius* pasca perlakuan cekaman merkuri. Sampel yang digunakan merupakan tanaman *A. palifolius* pasca perlakuan cekaman merkuri selama 14 hari pada bioreaktor FWS-CW. Dilakukan pengukuran respons fisiologis pertumbuhan, pengamatan jaringan anatomis, pengukuran parameter lingkungan, isolasi DNA, amplifikasi DNA, elektroforesis, Isolasi RNA, RT-PCR dan ekspresi gen. Karakter genetik dianalisis dengan kemurnian hasil isolasi DNA dan RNA. Hasil amplifikasi DNA dan amplikon qPCR divisualisasi menggunakan elektroforesis dan ekspresi gen dianalisis dengan metode *relative quantification*/ΔCq dengan normalisator total jumlah RNA. Gen GSH-s dipilih sebagai gen ketahanan berdasarkan *pathway* produksi fiotkelatin dalam sel. Penelitian menunjukkan bahwa terdapat tren kenaikan pada tiap parameter respons fisiologis pertumbuhan serta akumulasi merkuri pada aerenkim dan metaxilem *A. palifolius* pasca perlakuan merkuri. Deteksi gen yang dilakukan memerlukan optimasi namun dapat disimpulkan bahwa respons fisiologis pertumbuhan *A. palifolius* tidak terhambat dan gen GSH-s memiliki kemungkinan berperan besar sebagai gen ketahanan pada *A. palifolius*. Diketahui pula bahwa perlu diteliti lebih lanjut jalur/ *pathway* manakah yang dominan digunakan oleh *A. palifolius* dalam mengkelat merkuri.

KATA KUNCI: *Aquarius palifolius* (Nees & Mart.) Christenh. & Byng Bioremediasi, Deteksi Gen, Fitokelatin, Respons Fisiologis Pertumbuhan

PHYSIOLOGICAL RESPONSE AND DETECTION OF MERCURY TOLERANCE GENES IN *Aquarius palifolius* (Ness & Mart.) Christenh. & Byng

Mutiara Wahyu Nirmalasari

20/458309/BI/10542

Supervisor: Dwi Umi Siswanti, S.Si.,M.Sc.

ABSTRACT

Mercury waste from small-scale artisanal gold mining is a major source of heavy metal waste. Mercury waste that lacks proper handling will lead to environmental and health issues. Thus, bioremediation efforts are required to lessen the toxicity of mercury in the environment. Bioremediation with the plant agent *Aquarius palifoliu*s has been shown to be efficient in lowering mercury toxicity via the phytochelatin production pathway. The ability of *A. palifolius* as a hyperaccumulator plant can be reviewed based on its physiological growth responses (number of leaves, height, and diameter of the leaf stalk), anatomical tissue observation as well as the detection of tolerance genes that regulate the synthesis of phytochelatin. The objective of this research is to analyze physiological growth response, the location of the mercury inside the anatomical tissue and also to find the tolerance genes in *A. palifolius* under mercury stress. *A. palifolius* plants that had been exposed to a 14-day mercury stress treatment in an FWS-CW bioreactor were used as the samples. The procedures included physiological growth response measurements, anatomical tissues observation, RNA isolation, RT-PCR, electrophoresis, gene expression analysis, DNA amplification, and DNA isolation. The purity of the DNA and RNA isolation were used to assess the genetic characterization. Using total RNA as the normalizer, the relative quantification/ΔCq method was used to examine gene expression, and electrophoresis was used to visualize the amplicons obtained from qPCR and DNA amplification. The GSH-s gene was selected as the tolerance gene because it regulates the amount of GSH (glutathione), which is used in phytochelatin synthesis. Research shows that there is some mercury accumulation at the aerenchyma and the metaxylem, followed by a growth trend in each growth parameter after mercury stress. Hence the physiological growth responses of *A. palifolius* were not inhibited and the GSH-s gene has a big possibility of being the tolerance genes. Additional research will be needed to discover the primary pathway that *A. palifolius* uses for mercury chelation.

KEY WORDS: *Aquarius palifolius* (Nees & Mart.) Christenh. & Byng, Bioremediation, Gene Detection, Physiological Growth Response, Phytochelatins.