RINGKASAN

Ceriops tagal merupakan anggota familia Rhizophoraceae yang biasanya tumbuh pada hutan mangrove bagian dalam pada daerah tergenang oleh pasang tinggi. Spesies ini dapat ditemukan di beberapa kawasan hutan mangrove di sekitar Pulau Jawa, seperti Taman Nasional Kepulauan Seribu, Taman Nasional Karimunjawa, Segara Anakan Cilacap, dan Taman Nasional Baluran. Terdapat perbedaan karakteristik ekologi di antara beberapa hutan mangrove tersebut, yang dapat mempengaruhi adaptasi tumbuhan di dalam ekosistem tersebut. Informasi mengenai adaptasi *C. tagal* terhadap lingkungannya, terutama pada populasi di Pulau Jawa dan sekitarnya masih sangat terbatas. Selain itu, juga perlu dikaji mengenai karakter fisiologi, morfologi, dan anatomi *C. tagal* yang lebih spesifik terkait dengan adaptasinya pada lingkungan yang berbeda secara ekologi. Penggunaan marka molekuler yang akurat juga dibutuhkan untuk memprediksi perbedaan genetik di antara populasi *C. tagal*. Penelitian ini bertujuan untuk (1) mengevaluasi variasi fisiologi *C. tagal* berdasarkan konsentrasi logam, kadar klorofil, dan aktivitas enzim SOD (superoksida dismutase) dalam bagian tumbuhan; (2) mengevaluasi karakter morfo-anatomi *C. tagal* dari empat lokasi berbeda secara ekologi; (3) mengevaluasi variasi genetik *C. tagal* berdasarkan marka ITS.

Sampel tumbuhan *C. tagal* dalam penelitian ini berasal dari empat lokasi, yaitu Taman Nasional Kepulauan Seribu (Pulau Penjaliran Timur 5°27’53,26” LS 106º33’10,13” BT), Taman Nasional Karimunjawa (Pulau Kemujan 5º49’39,8” LS 110º28’36,0” BT), Taman Nasional Baluran (Kalitopo 7º50’26,1” LS 114º27’46,4” BT dan Popongan 7º51’59,24” LS 114º27’26” BT), dan Segara Anakan Cilacap (bagian timur 7º43’38,5” LS, 108°59’2,6’’ BT). Pengukuran faktor lingkungan meliputi suhu air, suhu tanah, suhu udara, pH air, pH tanah, salinitas air, dan intensitas genangan (inundasi).

Pengukuran kandungan logam (logam berat Pb, Cd, dan mineral mikro Cu, Zn, Fe, Mn) dalam air, sedimen, daun, ranting dan akar dilakukan menggunakan metode AAS (*Atomic Absorption Spektroscopy*). Pengukuran parameter fisiologi, meliputi aktivitas enzim SOD pada sampel *C. tagal* (akar, ranting, daun), dilakukan menggunakan *RANSOD Manual Randox Laboratories* dengan modifikasi, dan absorbansi dilihat pada λ 520 nm. Kandungan klorofil daun diukur absorbansinya pada λ 649 nm dan 665 nm.

Karakter morfologi meliputi panjang internodus, daun, dan tangkai daun, serta lebar, dan luas daun. Pengukuran karakter stomata meliputi kerapatan stomata, panjang stoamata, dan lebar stomata. Parameter anatomi akar yang diamati meliputi diameter akar, diameter silinder pembuluh (stele), diameter empulur, tebal epidermis, tebal hipodermis, tebal korteks, tebal floem, dan ketebalan xilem. Parameter anatomi ranting yang diamati meliputi diameter ranting, diameter silinder pembuluh (stele), diameter empulur, tebal kutikula, tebal epidermis, tebal hipodermis, tebal korteks, tebal endodermis, tebal floem, tebal kambium, dan tebal xilem. Parameter anatomi daun yang diamati meliputi tebal daun, tebal kutikula, tebal epidermis adaksial, tebal hipodermis, tebal palisade, tebal bunga karang, dan tebal epidermis abaksial.

Ekstraksi DNA dilakukan menggunakan GENEAID Genomic DNA Mini Kit. Amplifikasi DNA dilakukan menggunakan primer ITS 5P (5’ GGA AGG AGA AGT CGT AAC AAGG 3’) dan 8P (5’ CAC GCT TCT CCA GAC TACA 3’), pada kondisi suhu *initial denaturation* 94ºC- 3 menit, 35 siklus (*denaturation* 94ºC- 1

menit, *annealing* 55 ºC- 1 menit, *elongation* 72ºC- 1 menit), *final elongation* 72ºC- 5 menit, dan *holding* pada suhu 12ºC. Hasil PCR dikirim ke Firstbase Singapura untuk dipurifikasi dan sekuensing.

Analisis data variasi fisiologi meliputi *bio-concentration faktor* (BCF), *translocation faktor* (TF), hubungan antara konsentrasi logam dan faktor lingkungan dengan klorofil daun *C. tagal* menggunakan uji *pearson correlation* pada *software* PAST 4.10. Hubungan dan pengaruh konsentrasi logam dan faktor lingkungan dengan aktivitas enzim SOD pada akar, ranting dan daun *C. tagal* dianalisis menggunakan *Partial Least Square*. Pengukuran karakter morfologi dilakukan menggunakan *software* ImageJ, analisis variasi morfologi dilakukan menggunakan *principal component analysis* (PCA) pada *software* PAST 4.10. Hubungan dan pengaruh antara konsentrasi logam dan faktor lingkungan dengan karakter morfologi daun dan ranting *C. tagal* dianalisis menggunakan *Partial Least Square*. Pengukuran karakter anatomi dilakukan menggunakan *software ImageJ*, analisis variasi anatomi dilakukan dengan uji *duncan multiple range test* (DMRT) pada taraf signifikansi 0,05 dalam *software* SPSS versi 22. Pengelompokan berdasarkan karakter anatomi dilakukan menggunakan *principal component analysis* (PCA) pada *software* PAST 4.10. Hubungan dan pengaruh antara konsentrasi logam dan faktor lingkungan dengan karakter anatomi akar, ranting, dan daun *C. tagal* dianalisis menggunakan *Partial Least Square*. Penjajaran sekuen DNA diakukan menggunakan *software* BioEdit. Pohon filogenetik dikonstruksi menggunakan metode Neighbor-Joining dengan uji *bootstrap* 1000 ulangan, dan jarak genetik dihitung menggunakan metode *p-distance* pada *software* MEGA X. Variasi genetik dalam dan antarpopulasi dianalisis menggunakan *analysis of molecular variance* (AMOVA) pada *software* Arlequin 3.5.

Hasil penelitian menunjukkan bahwa aktivitas enzim SOD dipengaruhi oleh beberapa faktor lingkungan yang menyebabkan adanya cekaman dan memicu terbentuknya ROS, yaitu tekstur berupa pasir, genangan, salinitas air, dan suhu sedimen. Logam Cd dan Pb juga menyebabkan cekaman yang memicu terbentuknya ROS. Selain itu, rasio logam-logam Cu, Zn, Mn, dan Fe yang berfungsi sebagai kofaktor dalam aktivitas enzim SOD juga menjadi faktor yang berpengaruh terhadap naik atau turunnya aktivitas enzim SOD. Lima faktor lingkungan yang simultan, yaitu tekstur liat, tekstur debu, suhu udara yang relatif normal, pH tanah dan PH air yang relatif normal memberikan dukungan pada kondisi fisiologi, yaitu kandungan klorofil *C. tagal* yang lebih tinggi, ukuran morfologi daun yang lebih besar, kerapatan stomata daun *C. tagal* yang lebih tinggi, pori stomata yang relatif pendek, daun yang lebih tebal, serta ukuran anatomi akar dan ranting yang relatif kecil.

Lima faktor lingkungan yang juga simultan, yaitu suhu tanah dan suhu air yang relatif tinggi, salinitas air yang relatif tinggi, tekstur berupa pasir, dan genangan yang relatif jarang atau kondisi kekeringan, memberikan dampak yang sebaliknya, yaitu kandungan klorofil daun *C. tagal* yang cenderung rendah, morfologi daun yang lebih kecil, daun yang cenderung lebih tipis, stomata daun yang relatif panjang tetapi kerapatannya rendah, serta ukuran anatomi akar dan ranting yang relatif lebih besar.

Efek logam pada struktur anatomi pada bagian akar dan ranting *C. tagal* terlihat berupa bintik hitam yang menandakan adanya akumulasi logam pada jaringan, sedangkan efek logam pada anatomi daun adalah bentuk palisade yang relatif tidak teratur. Pengaruh langsung baik faktor lingkungan maupun logam pada variasi genetik *C. tagal* dalam penelitian ini tidak terlihat. Tetapi, Taman Nasional

Baluran memiliki sekuen ITS yang berbeda dengan lokasi lain. Hal ini karena adaptasi terhadap lingkungan yang ekstrim membuat struktur genetik *C. tagal* di Taman Nasional Baluran menjadi unik, sejalan dengan perubahan fisiologi, morfologi, dan anatominya, sebagai rangkaian adaptasi terhadap lingkungan yang ekstrim.

Kata kunci: *Ceriops tagal*, klorofil, morfo-anatomi, superoksida dismutase, variasi genetik

SUMMARY

Ceriops tagal is a member of the Rhizophoraceae family, which typically develops in the inner mangrove forests in places that experience high tide inundation and possess well-drained soils. This species can be found in several mangrove forest areas around Java, such as the Kepulauan Seribu National Park, Karimunjawa National Park, Segara Anakan Cilacap, and Baluran National Park. Some mangrove forests in the regions have different ecological traits, which may have an impact on the adaptability of plants in the ecosystem. There is currently very little known about how *C. tagal* populations, particularly those in Java Island, have adapted to their habitats. Additionally, there haven't been any reports of more detailed investigations on the physiological, morphological, and anatomical traits of *C. tagal* in relation to its adaptability to ecologically various habitats. To determine the genetic variations among *C. tagal* populations, suitable molecular markers are also required. The objectives of this study are to: (1) evaluate the physiological variation of *C. tagal* based on the metal concentration, chlorophyll content, and SOD enzyme activity in plant parts; (2) evaluate the morpho-anatomical characteristics of *C. tagal* from four ecologically distinct locations; and (3) evaluate the genetic variation of C. *tagal* based on ITS markers.

C. tagal Plant samples were taken from four locations, i.e. the Kepulauan Seribu National Park (Penjaliran Timur Island 5°27'53.26" S 106º33'10.13" E), Karimunjawa National Park (Kemujan Island 5°49'39.8" S 110º28' 36.0" E), Baluran National Park (Kalitopo 7º50'26.1" S 114º27'46.4" E and Popongan 7º51'59.24" S 114º27'26" E), and Segara Anakan Cilacap (eastern part 7º43'38.5" S, 108°59'2.6" E). Sampling locations were determined purposively by considering the presence of *C. tagal* and the ecological conditions of each location. Measurement of environmental factors included water temperature, soil temperature, air temperature, water pH, soil pH, water salinity, and inundation.

The AAS (atomic absorption spectroscopy) method was used to determine the metal content of (heavy metals Pb, Cd, and trace elements Cu, Zn, Fe, and Mn) in water, sediment, leaves, twigs, and roots. Physiological parameter measurements included SOD enzyme activity in *C. tagal* samples (roots, twigs, and leaves) using the RANSOD Manual Randox Laboratories with modifications, and absorbance was measured at λ 520 nm. The absorbance of leaf chlorophyll was measured at λ 649 nm and 665 nm.

Morphological characters included the length of the internodes, leaves, and petioles, as well as the width and area of the leaves. Stomata character measurements included stomata density, stomata length, and stomata width. Root anatomical parameters observed included root diameter, stele diameter, pith diameter, epidermal thickness, hypodermis thickness, cortex thickness, phloem thickness, and xilem thickness. The anatomical parameters of the twigs observed included twig diameter, stele diameter, pith diameter, cuticle thickness, epidermal thickness, hypodermis thickness, cortex thickness, endodermis thickness, phloem thickness, cambium thickness, and xilem thickness. Leaf anatomy parameters observed included leaf thickness, cuticle thickness, adaxial epidermal tissue thickness, hypodermis thickness, palisade tissue thickness, spongy tissue thickness, and abaxial epidermal tissue thickness.

DNA extraction was performed using the GENEAID Genomic DNA Mini Kit. DNA amplification was made using primers ITS 5P (5' GGA AGG AGA AGT CGT AAC AAGG 3') and 8P (5' CAC GCT TCT CCA GAC TACA 3'), at an initial

denaturation temperature of 94ºC for 3 minutes, 35 cycles (denaturation 94ºC for 1 minute, annealing 55 ºC for 1 minute, elongation 72ºC for 1 minute), and a final elongation 72ºC for 5 minutes, and maintained at 12ºC. PCR products were sent to Firstbase Singapore for purification and sequencing.

Data on physiological variation, including bio-concentration factor (BCF), translocation factor (TF), and the relationship between metal concentrations and environmental factors with leaf chlorophyll were analyzed using the Pearson correlation test in PAST 4.10 software. The relationship and influence of metal concentrations and environmental factors on SOD enzyme activity in the roots, twigs, and leaves of *C. tagal* were analyzed using Partial Least Square. Morphological characters were measured using ImageJ software, while analysis of morphological variation was performed using principal component analysis (PCA) in PAST 4.10 software. The relationship and influence of metal concentrations and environmental factors on the morphological characters of *C. tagal* leaves and twigs was analyzed using Partial Least Square. Anatomical characters were measured using ImageJ software, while analysis of anatomical variation was performed using the Duncan multiple range test (DMRT) at a significance level of 0.05 in SPSS software version 22. Grouping based on anatomical characters was performed using principal component analysis (PCA) in PAST 4.10 software. The relationship and influence of metal concentrations and environmental factors on the anatomical characters of roots, twigs and leaves of *C. tagal* was analyzed using Partial Least Square. DNA sequence alignment was performed using BioEdit software. Phylogenetic trees were constructed using the Neighbor-Joining method with a bootstrap test of 1000 replications, and genetic distances were calculated using the p-distance method in MEGA X software. Genetic variations within and between populations were analyzed using analysis of molecular variance (AMOVA) in Arlequin 3.5 software.

The results show that SOD enzyme activity is influenced by several environmental factors that cause stress and trigger the formation of ROS, namely texture in the form of sand, puddles, water salinity, and sediment temperature. Cd and Pb metals also cause stress, which triggers the formation of ROS. In addition, the ratio of Cu, Zn, Mn, and Fe metals, which function as cofactors in SOD enzyme activity, is also a factor that influences the increase or decrease in SOD enzyme activity. Five simultaneous environmental factors, namely clay texture, dust texture, relatively average air temperature, and relatively normal soil pH and water pH, provide support for physiological conditions, namely higher chlorophyll content in *C. tagal*, more extensive leaf morphology, and density *C. tagal* leaves have more stomata, relatively short stomatal pores, thicker leaves, and relatively small root and twig anatomy. Conversely, the opposite five environmental factors, namely relatively high soil and water temperatures, relatively high water salinity, sandy texture, and relatively periodic inundation or drought conditions, have the opposite effect, namely lower chlorophyll content of *C. tagal* leaves, relatively narrower leaf morphology, thinner leaves, relatively longer leaf stomata but with lower density, and relatively larger anatomical sizes of rooster.

The effect of metal on the anatomical structure of the roots and branches of *C. tagal*, namely the presence of black spots, indicates the accumulation of metal in the tissue, while the effect of metal on the anatomy of the leaves is the relatively irregular palisade shape. The direct influence of environmental factors and metals on the genetic variation of *C. tagal* in this study was not seen, but Baluran National Park has ITS sequences that are different from other locations. This is because the adaptation to extreme environmental conditions has made the genetic structure of *C.*

tagal in Baluran National Park unique, along with changes in its physiology, morphology, and anatomy as a result of a series of adaptations to extreme environments.

Key words: *Ceriops tagal*, chlorophyll, genetic variation, morpho-anatomy, superoxide dismutase