ABSTRAK

RESPON FISIOLOGIS DAN EKSPRESI GEN–GEN *HSP 81-2, HSP 70, P5CS1* DAN *PAL* PADA SOMAKLON PISANG (*Musa acuminata* Colla) DALAM KONDISI CEKAMAN SALINITAS *IN VITRO*

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Pisang (*Musa spp*) merupakan salah satu jenis buah yang memiliki nilai penting ditinjau dari sudut kandungan nutrisi yang dimilikinya, buah ini kaya dengan karbohidrat, vitamin dan mineral. Buah pisang menjadi komoditas ekspor utama di beberapa negara tropis dan sub tropis. Berbagai upaya peningkatan produksi pisang telah banyak dilakukan, antara lain melalui perluasan area penanaman. Walaupun demikian, upaya tersebut seringkali terkendala oleh adanya berbagai cekaman lingkungan, terutama cekaman abiotik. Di antara berbagai jenis cekaman lingkungan, salinitas menjadi salah satu cekaman abiotis paling penting yang dapat mempengaruhi pertumbuhan dan secara langsung dapat mempengaruhi produktivitas tanaman. Pisang termasuk jenis tanaman mesofit yang tidak tahan terhadap salinitas tinggi. Produktivitas tanaman pisang yang ditanam di lingkungan dengan tingkat salinitas tinggi akan mengalami penurunan hingga 50%. Upaya untuk mendapatkan pisang yang tahan terhadap cekaman salinitas telah dilakukan oleh beberapa peneliti termasuk tentang regulasi dan mekanisme pertahanan untuk mendapatkan tanaman pisang yang toleran terhadap salinitas tinggi. Cekaman salinitas dapat mempengaruhi gen yang berperan dalam beberapa proses pertahanan antara lain gen *HSP 81-2* maupun *HSP 70* yang mengekspresikan protein *chaperone*, gen yang terlibat dalam proses metabolisme nitrogen (*P5CS1*) yang mengekspresikan enzim pirolin-5-karboksilat sintase dan gen yang terlibat dalam proses sintesis lignin (*PAL*) yang mengekspresikan enzim fenilalanin ammonia-liase. Penelitian ini dilakukan dengan tujuan untuk mengevaluasi toleransi somaklon pisang (*Musa acuminata* Colla) cv. Barangan yang terseleksi toleran terhadap cekaman salinitas. Kemampuan toleransi ini dievaluasi berdasarkan pertumbuhan, analisis biokimia (kadar klorofil, karotenoid dan prolin) dan analisis molekuler (pola ekspresi dari gen-gen *HSP81-2, HSP 70, P5CS1* dan *PAL* serta keterkaitan antar gen tersebut. Eksplan yang digunakan adalah galur somaklon pisang Barangan hasil seleksi terhadap cekaman NaCl. Seleksi dilakukan dengan mendedahkan pucuk pada medium Murashige dan Skoog (MS) dengan penambahan 25, 50, 75 dan 100 mM NaCl secara suksesif dan kemudian dilakukan pengujian toleransi. Seleksi ini dilakukan untuk mendapatkan somaklon toleran (T). Sebagai pembanding adalah pucuk yang dikultur dalam medium MS0 tanpa penambahan NaCl (NTo) dan pucuk yang diberi cekaman NaCl secara langsung (NT). Parameter pertumbuhan ditunjukkan dengan pertambahan jumlah dan ukuran pucuk dan/atau pertambahan jumlah dan ukuran daun. Gejala kematian ditunjukkan dengan daun yang mengalami nekrosis. Analisis biokimiawi dilakukan dengan melakukan pengukuran kadar klorofil, karotenoid dan prolin secara

spektrofotometri. Analisis pola ekspresi gen-gen *HSP 81-2, HSP 70, P5CS1* dan *PAL* dilakukan secara *in silico* dengan menggunakan pendekatan *qRT-PCR*. Pola ekspresi dianalisis lanjut tentang keterkaitannya berdasarkan heatmap dan korelasi Spearman menggunakan *software GenEx*. Sekuen gen diperoleh dari analisis data transcriptome pucuk pisang Barangan yang terkena cekaman NaCl milik KK SBT SITH ITB. Primer gen didesain dengan software Primer3 menggunakan parameter default dari software tersebut. Hasil penelitian menunjukkan bahwa pertumbuhan pada somaklon NT100 sangat rendah (4%) dengan gejala kematian yang tinggi (82%) sedangkan pada somaklon T100 pertumbuhannya mencapai 72% dengan gejala kematian yang rendah (8%). Kadar klorofil a, b, total dan karotenoid lebih rendah dibanding NTo kecuali klorofil b dan karotenoid pada somaklon T25. Kadar prolin pada pucuk dan akar somaklon NT100 lebih tinggi dibanding somaklon NTo dan T. Dari data pertumbuhan dan biokimia mendukung bahwa adanya keterkaitan antara pertumbuhan dan kandungan komponen fotosintesis serta prolin. Pola ekspresi gen menunjukkan gen-gen *HSP 81-2, HSP 70, P5CS1* dan *PAL* diekspresikan dengan level berbeda pada daun maupun akar setiap somaklon. Hasil analisis lanjut dari level ekspresi menunjukkan pada somaklon T100 terdapat keterkaitan antara gen *HSP 81-2* dengan gen *HSP 70*, gen PAL dengan gen *HSP 70* dan gen *P5CS1* dengan gen *PAL*. Penelitian ini menunjukkan bahwa berdasarkan respon fisiologis dan analisis molekuler pada somaklon pisang (*Musa acuminata* Colla) cv. Barangan yang terseleksi toleran terhadap cekaman salinitas memiliki kemampuan dalam mengatasi cekaman salinitas ditunjukkan dengan cara mengaktifkan jalur pertahanan yang melibatkan gen-gen *HSP 81-2, HSP 70, P5CS1* dan *PAL*. Aktivitas gen-gen tersebut saling terkait dan kemungkinan saling mengontrol satu sama lain. Kandungan klorofil dan karotenoid lebih tinggi dan kandungan prolin lebih rendah pada somaklon T100 dibanding somaklon NT100. Pada somaklon toleran, gen yang terinduksi adalah gen *HSP 70* dan gen *P5CS1* sehingga keduanya dapat digunakan sebagai gen kunci dalam toleransi terhadap adanya cekaman salinitas pada pisang cv. Barangan. Hasil penelitian ini telah membuka jalur baru penelitian terkait dengan gen-gen faktor transkripsi yang terlibat dalam toleransi terhadap cekaman salinitas pada pisang (*Musa acuminata* Colla) cv. Barangan, karena pada penelitian ini sudah dilakukan pada gen-gen regulator. Penelitian lain tentang keterkaitan antara gen faktor transkripsi dan gen regulator lain berdasarkan data transcriptome. Dengan mengetahui respon fisiologis dan pola ekspresi gen petahanan tersebut maka dapat dilakukan upaya untuk mendapatkan tanaman pisang yang toleran yang dapat ditanam di lahan-lahan marginal sehingga diharapkan dapat mengatasi pemanfaatan lahan marjinal dengan peningkatan produksi pisang.

Kata Kunci : Salinitas, Pertumbuhan, Klorofil, Prolin, Pola ekspresi, Keterkaitan

ABSTRACT

PHYSIOLOGICAL RESPONSES AND HSP 81-2, HSP 70, P5CS1, PAL GENE EXPRESSIONS IN BANANA (Musa acuminata Colla*) SOMACLONE UNDER IN VITRO SALT STRESS CONDITIONS*

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Banana (Musa acuminata L.*) is a type of fruit based on nutritional value. This fruit is rich in carbohydrates, vitamins and minerals. Bananas are considered as major exported fruit commodity in several tropical and sub tropical countries. Efforts to increase the production of bananas has been widely done, i.e through the expansion of banana plantation areas. Land expanding, however, is often constrained by the various environmental stresses. Among the various types of abiotic stress, salinity becomes one of the most important abiotic stresses which can directly affects plant growth and productivity. Banana is mesophytic plant that intolerant to high salinity therefore growing banana in the environments with high salinity levels will reduce productivity, even up to 50%. Some researchers have attempted to obtain bananas that are resistant to salinity stress, including research on regulation and defense mechanisms to obtain high salinity tolerance bananas. Salinity can affect genes that are involved in some defense processes this include HSP 81-2 and HSP 70 genes that express the chaperone protein, the genes involved in the nitrogen metabolism (P5CS1) process that express the pyrroline-5-carboylate synthase enzyme and gene involved in the lignin synthesis process (PAL) that express the phenylalanine ammonia-lyase enzyme. The aim of this study was to evaluate the tolerance of banana (Musa acuminata* Colla*) somaclone cultivar barangan that was selected tolerant to salinity stress. This tolerance capability is evaluated by growth rate, biochemical analysis (chlorophyll, carotenoid and proline content) and molecular analysis (expression patterns of HSP81-2, HSP 70, P5CS1 and PAL genes) and the pattern of expression relationships between these genes. The explants used in this research are Barangan banana somaclone strain which is the result of selection against NaCl stress. This selection was performed by exposing the shoots in Murashige and Skoog (MS) mediums by adding 25, 50, 75 and 100 mM NaCl successively and then testing tolerance treatment. This selection was performed to obtain a tolerant somaclone (T). As a comparison in this selection the shoots were cultured in MS medium without the addition of NaCl (NTo) and shoot cultured in MS medium which was given NaCl stress directly (NT). The growth parameters measured in this study were the rate of increase in the number and size of shoots and / or the rate of increase in the number and size of the leaves. Symptoms of death that occur is shown by leaves that have necrosis. Biochemical analyzes were performed by measuring chlorophyll, carotenoid and proline levels by spectrophotometry.*

Analysis of expression pattern of HSP 81-2, HSP 70, P5CS1 and PAL genes were done in silico using qRT-PCR approach. The expression pattern were further analyzed on its association based on the heatmap and Spearman correlation using GenEx software. The gene sequences used in this study were sequences obtained from transcriptome data analysis of shoots of banana cv. Barangan affected by NaCl stress belonging to KK SBT SITH ITB. *Primary genes used in this study were designed with Primer3 software using the default parameters of the software. The results showed that the growth rate in somaclones NT100 was very low (4%) with high mortality rate (82%) whereas in T100 somaclone the growth rate reached 72% with low mortality rate (8%). The content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid in somaclone treatment was lower than that of NTo except the content of chlorophyll b and carotenoid in T25 somaclone was higher than NT25. The proline level on shoots and roots in the NT100 somaclone is higher than for NTo and T somaclones. Based on data the growth rates and biochemical analysis in this study supports evidence that there was a link between growth rates and photosynthetic component and proline contents. The pattern of gene expression showed the genes of HSP 81-2, HSP 70, P5CS1 and PAL expressed with different levels of leaf and root samples in each somaclone. Further analysis of the expression level indicated that on the somaclone T100 there was an association of expression between HSP 81-2 and HSP genes 70, PAL with HSP 70 genes and P5CS1 with PAL genes. This study shows that based on physiological response and molecular analysis data on Barangan banana somaclone which is a plant tolerant to salinity stress has the ability to overcome the stress of salinity shown by activating defense pathways involving HSP 81-2, HSP 70, P5CS1 and PAL genes. The activities of these genes were interrelated and there was the possibility of controlling each other. The content of chlorophyll and carotenoid T100 was higher than that of NT100 and the proline content was lower on T100 than NT100 somaclone. In tolerable somaclones, the induced genes are the HSP 70 and P5CS1 genes so that they can be used as key genes in the tolerance of salinity stability in banana cv. Barangan. The results of this study have opened up new pathways associated with transcriptional factor genes involved in tolerance of salinity in bananas (Musa acuminata* Colla*) cv. Barangan, because in this study that has been done only on the genes of the regulator. Another study of the relationship between transcriptional factor and other regulatory genes based on transcriptome data has been done. By knowing the physiological responses and expression patterns of these defense genes as an attempt to obtain tolerant banana crops that can be planted on marginal lands is expected to overcome the utilization of marginal land through increased banana production.*

Keywords: Salinity, growth rate, Chlorophyll, Proline, Expression Pattern, Interrelationship Expressional Pattern