

**FINAL PROJECT REPORT**

**MECHANISM OF GENE REGULATION BY  
MICRORNAS AT DIFFERENT  
DEVELOPMENTAL STAGES OF FRUIT  
RIPENING**

**FRG0164-SG-2008**

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## ABSTRACT

### **Mechanism of gene regulation by microRNAs at different developmental stages of fruit ripening**

Pineapple (*Ananas comosus* var. *comosus*) is a non-climacteric fruit, of which the underlying mechanism of fruit ripening is still unknown. The non-ethylene induced ripening of non-climacteric fruit is very much different from climacteric ripening as revealed by the model organism of flesh-fruit i.e. tomato. Recently discovered gene regulators, i.e. miRNAs, are known to silence the translation of mRNA transcripts, leading to a silencing effect on proteins and affecting various phenotype changes in plants throughout plant development. Thus, this study aims to determine the mechanism of non-climacteric ripening through the differential expression of miRNA genes. Orthologous miRNAs were first amplified through stem-loop RT-PCR to prove for the existence of the presence of miRNAs in pineapples. Large scale mining of miRNA from small RNA libraries (fruit and leaf) constructed through the use of high-throughput Solexa technology was then carried out and the target mRNA transcripts were subsequently predicted. For this purpose, the miRNA registry (miRBase) and EST database (NCBI) were referred to and a bioinformatics pipeline was developed and utilized. The regulation of miRNAs in regulation of fruit ripening was postulated through correlating the target protein predicted with the pattern of expression of miRNAs during pineapple ripening obtained through stem-loop RT-qPCR. As a result, 12 orthologous miRNAs were amplified and showed the presence of miRNAs in pineapple, while finding of 153 miRNAs, 41 miRNA families, 20 miRNA\*s, and 20 target transcripts obtained showed the conservation of miRNAs in pineapple. The miRNAs were then characterized. Finally, differential regulation was postulated where 7 miRNAs (miR165, miR166, miR164, miR171, miR444, miR1088, and miR396) are believed to be involved in fruit development and 4 miRNAs (miR172, miR156, miR535, miR319) in phase transition from flowering to senescence. Besides that, throughout pineapple development, 5 miRNAs (miR159, miR167, miR390, miR393 and miR394) control hormone signaling, 4 miRNAs (miR395, miR397, miR399, and miR893) control nutrient uptake and homeostasis, and 2 miRNAs (miR162 and miR168) control the overall homeostasis of miRNAs. Thus, the preliminary putative of pineapple regulated by miRNAs is postulated and revealed.



## ABSTRAK

*Nenas (Ananas comosus) berada dalam kategori buah bukan klimakterik dimana mekanisme pemasakannya masih tidak diketahui. MiRNA memainkan peranan dalam menghalang penterjemahan gen-gen lain kepada protein dengan menasarkkan transkrip-transkrip untuk dihancurkan atau disekat dari diterjemahkan kepada protein, lalu meyebabkan perubahan fenotip pada tumbuhan. Oleh itu, kajian ini adalah bertujuan mempelajari pemasakan buah bukan-klimakterik melalui miRNA. Ortolog miRNA pada mulanya diamplifikasi bagi membuktikan kewujudan miRNA pada nenas menggunakan kaedah stem-loop RT-PCR. Kajian diteruskan dengan pengenalpastian miRNA dari perpustakaan sRNAs (daun dan buah) yang dijujuk menggunakan teknologi berimpak tinggi yang dipanggil Solexa. MiRNA dikenalpasti dari sRNA dengan membandingkan jujukan sRNAs dengan jujukan miRNA yang terdapat dalam pusat pengumpulan miRNA (miRBase) dan ia kemudiannya dibandingkan pula dengan jujukan EST dan protein yang terdapat di NCBI bagi tujuan pengenalpastian transkrip dan protein sasarannya. Kaedah bioinformatik diaplikasikan. Corak ekspresi bagi miRNA yang baru dijumpai semasa dua peringkat pemasakan buah (pre-pemasakan dan pasca-pemasakan) telah dibandingkan dengan menggunakan kaedah stem-loop RT-qPCR. Hasilnya, amplifikasi 12 ortolog miRNA membuktikan kehadiran miRNA pada nenas. Selain itu, penemuan 153 miRNA, 41 keluarga miRNA, 20 miRNA\*, dan 20 transkrip yang disasarkan oleh miRNA menunjukkan bahawa miRNA adalah terpuhara dalam nenas. MiRNA yang dijumpai telah dikaji dan dicirikan. Akhir sekali 7 miRNA (miR165, miR166, miR164, miR171, miR444, miR1088, and miR396) dipercayai terlibat dalam pembentukan buah, dan 4 miRNA (miR172, miR156, miR535, miR319) terlibat dalam peralihan fasa nenas bermula dari pertumbuhan bunga hingga peranuman buah. Selain itu 5 miRNA (miR159, miR167, miR390, miR393 and miR394) dipercayai mengawal hormon di sepanjang proses pembentukan buah nenas manakala 4 miRNA (miR395, miR397, miR399, and miR893) mengawal pengambilan dan keseimbangan nutrient pada nenas. Akhir sekali 2 miRNA (miR162 dan miR168) terlibat dalam pengawalan keseluruhan rangkaian pemasakan nenas yang dikawal oleh miRNA dengan memnyeimbangkan kadar miRNA.*