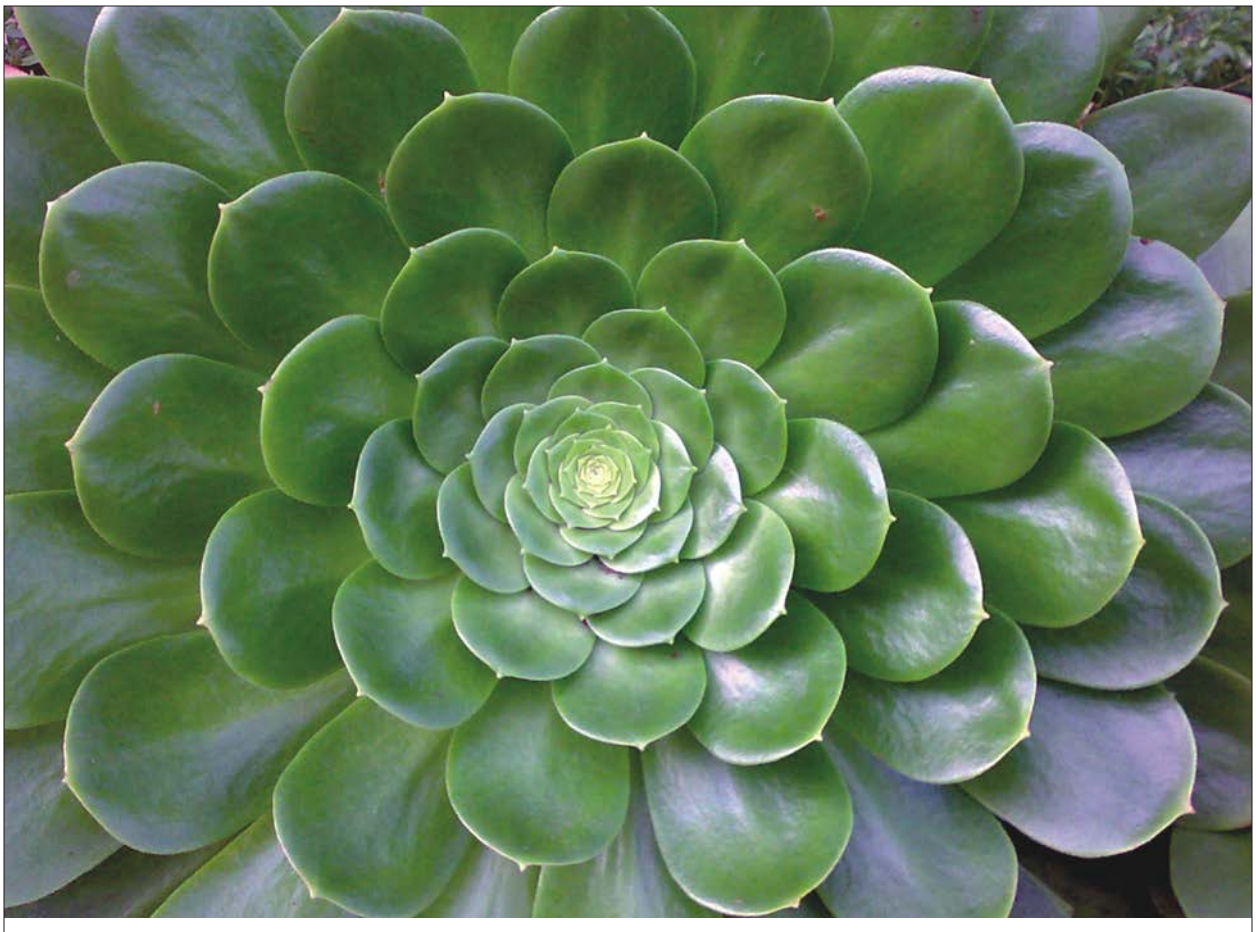

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Profile of a Coumarate-CoA Ligase-like5 Gene in Response to Salt Stress on the Root *Musa acuminata* 4-L. Barangan Cultivars

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ABSTRACT

Musa acuminata L. growth has influenced by environment. Environmental stresses are caused by biotic factors such as fungi, bacteria and herbivore and abiotic factors such as temperature, water, light and salinity. Salinity stresses cause a decrease in the production of banana. The purpose of the research was to validity and confirmation 4-Coumarate-CoA ligase Like5 (*4CLL5)* gene with 18S reference genes as a control genes on planlets of banana with sodium chloride (NaCl) application. 4-Caomarate-CoA-ligase Like5 is an enzyme key in phenyl-propanoid metabolism like lignin and flavonoid that essential for plant defense on abiotic factor like salinity is coded by *4CLL5* on *Musa acuminate* L. Salinity is abiotic factors which affect the growth of banana plant. Gene expression *4CLL5* on banana by using real-time quantitative polymerase chain reaction. Research using shoot banana *in vitro* culture with any concentration of sodium chloride (NaCl) were 0.00, 0.05, 0.10, 0.15 and 0.20 M. In addition to, in this research was using 4CLL5 as a primers. The expression of genes analysis was using real- time quantitative polymerase chain reaction. The results showed that *4CLL5* gene was expressed up regulated on NaCl 0.05 and 0.1 M but down regulated in 0.15 and 0.20 M.

Key words: Sodium chloride, *in vitro* culture, real-time quantitative polymerase chain reaction

INTRODUCTION

Salinity that result in the accumulation of dissolved salts in the soil that will inhibit plant growth and occurs naturaly or human induced (Yadav *et al*., 2011). Inadequate drainage of the fields results in the propressive accumulation of salt in the soil with the consequent decline in crop yield. The researcher has been able to compensate partially for loss yield. Mechanism by which tolerance is conferred remains unclear. To identity the primary effects of salt on plant growth and the biochemical adaptation. It might be possible to use molecular technology to know physiological processes in *Musa acuminata* L. for salinity affect.

The plants have secondary metabolites such as flavonoid, iso-flavonoid, sinapate esters and lignin. Lignin is end product of phenylpropanoid metabolism. Specific lignin from phenyl propanoid pathway such as ρ-coumaryl, coniferil and sinapyl alcohol monolignols, 4-Coumarate-CoA ligase (Baxter and Stewart Jr., 2013; Hu *et al*., 2008). The genes that is involved in lignin synthesis was 4-Coumarate CoA ligase like5 genes analysis was using real-time quantitative polymerase chain reactions (qRT-PCR).

Real-time quantitative polymerase chain reactions analysis to genes validity is used reference genes 18S, because it was more stable in different condition (Brunner *et al*., 2004). The primers was

found from transcriptome analysis *Musa acuminata* L. cv. Barangan which has been researched before in sodium chloride (NaCl) 0.1 M *in vitro* culture (Widiyanto *et al*., 2013). Transcriptome analysis had result ontology datas such as biology, moleculer and celuller process. Biology processes producing some metabolites like lignin and enzyme 4-Coumarate CoA Ligase Like5 (*4CLL5*). The purpose of the research was to validity and confirm of 4-Coumarate ligase-like5 (*4CLL5*) gene, 18S as reference gene with q RT-PCR analysis.

MATERIALS AND METHODS

The shoot of banana planlets from P.T. Multi Agro Kultura Pamulang Tangerang. The basic medium used were that of Murashige and Skoogs (MS), sugars, gel with Benzylaminopurine (BAP) 1.5 ppm as growth regulators and sodium chloride (NaCl) with various concentration 0.00, 0.05, 0.10, 0.15 and 0.20 M. This study was conducted since July 2014 to December 2014 in laboratory Molecular Cell Biology School of Life Science and Technology, ITB, Indonesia.

Culture of *Musa acuminata* L. *in vitro*: The shoot explant 3-4 week sub cultured using Murashige and Skoogs (MS), sugar, gel and Benzyl Ammino Purin (BAP) 1.5 ppm as growth regulator and add sodium chloride (NaCl) with various concentration 0.00, 0.05, 0.10, 0.15 and 0.20 M. Medium in the bottle sterilized in autoclaf. The explant cultured within steril medium, incubation for ±4 weeks. After 4 week harvested for the culture, shoot and root separated in an application. Shoot and root of explant (Fig. 1) were ground with nitrogen until powder and it using for RNA isolation.

Primer determination: Previously work has been researched on *Musa acuminata* L. Barangan cultivars with transcriptom analysis (Widiyanto *et al*., 2013). Transcriptome data obtained banana genomic from banana control and NaCl 0.10 M. The following genom was BLAST, annotation and ontology. It was found many processes such as biology, molecular and cellular. Gen interest from there was 4-Coumarate-CoA ligase-like5 or *4CLL5* gene (Widiyanto *et al*., 2013). The next step with Primer3 software was found 4CLL5 primers. 4-Coumarate-CoA ligase-like5 had a pairs of primers are: Left primer: GCAGCATACATCCCCAAGTT. Right primer: AGCAT

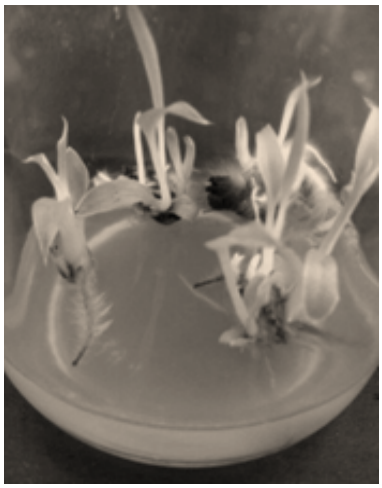

Fig. 1: *Musa acuminata* L. explant source

GCTTCCAGGTTGTCT. Reference genes using 18S, 18S genes more stable than others in different condition (Brunner *et al*., 2004). Primers of 18S are: Left primer: AGTTCTCTTCCAGCCATCCA Right primer: GCCGTGATTTCTTTGCTCAT.

RNA isolation: Buffer preparation, preheating in 65°C, the shoot and the root sample devided and ground in nitrogen 1.5 g. Add buffer 6 mL tube (15 mL tube). Incubate in 65°C for 30 min. Add chloroform; isoamylalcohol (24:v/v), vortex. After that centrifuges in 11000 grv for 15 min 4°C. The supernatants remove to the new tube and then the second extract equal volume of chloroform (24:1 v/v) then centrifuge. All of materials is mixed, incubated in ice for 2 h. Add 10 μL buffer 1.5 μL^{-1} from DNAse tube. After that resuspenses 500 μL, add SSTE buffer, incubated in 37°C for 30 min. Add chloroform: isoamylalcohol (24:1 v/v). Supernatans removed to the new tube. Add 0.7 volume isopropanols and centrifuges in 21000 grv for 15 min 4°C. After that washed the pellets with 70% ethanol (DEPC-H2), dry and resuspenses in RNAse free water-DEPC (Chang *et al*., 1993).

cDNA: RNA had been isolation was test using spectrometry machine and best quality of RNA is used qRTPCR analysis. The cDNA reagent is used in new tube made duplo. Reagent such as oligo random primers and specific gene. After that incubated 65°C for 5 min, add others component; buffer reaction 4 μL, Riboloc, RNAse inhibitor, dNTP 10 mM. All of materials mix and centrifuges, oligo primer and specific cDNA is incubated for 60 min at 42°C, then is terminated 70°C.

Real-time polymerase quantitative chain reaction: Real-time quantitative polymerase chain reaction (qRT-PCR) is the method for through put gene expression analysis and has improved the ease sensitivity of quantitative gene expression, validating the whole-genome microarray data and molecular diagnostics (Brunner *et al*., 2004; Jain *et al*., 2006).

Real-time quantitative RT-PCR producing full-length primers 4CLL5 used for amplification *4CLL5* with PCR site: template denaturation 95°C for 3 min, amplification 65°C for 1 min and extension 72°C for 1 min (Livak and Schmittgen, 2001; Singh *et al*., 2009).

RESULTS AND DISCUSSION

Coumarate CoA ligase Like-5 in *Musa acuminata* L.: Primers were designed with primer3 software of previous research by transcriptome analysis on *Musa acuminate* L. is affected salt stressed. It was found that 4-Coumarate CoA Ligase-Like 5 gene involved in phenylpropanoid biosynthesis, especially lignin synthesis. We used 18S housekeeping gene as references of gene. Expression of gene *4CLL5* and 18S gene reference were assessed by real-time PCR in a set of 5 samples with various concentration of sodium chloride. Olygodeoxythymidine (dT) and degenerate primers 4CLL5 based on protein sequence using qRT-PCR (Hu and Schmidhalter, 1998).

The high quality RNA total obtained from various quality controls have isolated from different application of 5 samples after spectrophotometry to determine RNA qualities. The high RNA quality showed by the absorbance value, whereas the best RNA quality absorbance values range 1.8-2.0. Within a biological replicate for sample, c-DNA pool was used for real-time PCR analysis of each 5 sample using reference gene as a control.

Gene 4-Coumarate CoA ligase Like5 expressed in various of sodium chloride concentration; 0.00, 0.05, 0.10, 0.15 and 0.20 M. Gene 4-Coumarate CoA ligase Like5 involved lignin biosynthesis

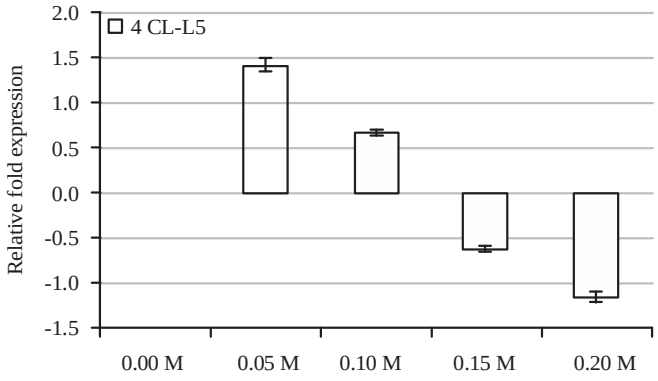

Fig. 2: Gene profile of *4CLL5* in the various concentration of sodium chloride on root planlets of *Musa acuminata* L.

pathway. Lignin is derived from monomers and three monolignols. The biosynthesis of the monolignols starts with the deamination of phenylalanine (Boerjan *et al*., 2003). Phenylalanine is catalyzed by Phenylalanine Ammonia Lyase (PAL) is an enzyme in branch point primer and secondary metabolism. It is key enzyme involve in phenylalanine pathway. Phenylalanine ammonium lyase has role resistensi modulation to abiotic stress (Rani *et al*., 2009; Alvarez *et al*., 2013; Baxter and Stewart Jr., 2013). Other enzyme involved in phenylalanine pathways are cinnamate 4-hydroxylase (CH4), 4-Coumarate CoA ligase (*4CL*) including 4-Coumarate CoA ligase like5 (Zhao *et al*., 2013). Gene encoding of 4CL, 4CLL5 are *4CL*, *4CLL5* . Gene *4CL* and *4CLL5* are family gene in other plant such as Arabidobsis thaliana, aspen (Hu and Schmidhalter, 1998).

Coumarate CoA ligase Like-5 expression profiles in the various of sodium chloride: In sodium chloride (NaCl) 0.05 M (Fig. 2) with relative fold expression values 5, after the expression level decreased in NaCl 0.15 and 0.20 M (down regulated). This is happened in NaCl 0.05 and 0.10 M showed that increased cell wall structure. Changed of lignin biosynthesis metabolism *4CLL5* related with stresses in plant is affected by the biotic factors as pathogen infection, herbivore or the abiotic factor by environment factor including salinity (Baxter and Stewart Jr., 2013).

Content of change of lignin in plant is affected stresses indirectly lignin metabolism pathway with genes expression (Dauwe *et al*., 2007). In this research showed that 4-Coumarate-CoA ligase like-5 more decreased related stressed. In this case, the structure cells wall plants damaged with stresses increased, event in the highest stressed cells death.

CONCLUSION

In conclusion, it have validated and identified 4-Coumarate CoA Ligase Like-5 genes and a housekeeping *18S* genes. The research allowed that the various of sodium chloride affected to gene expression profile of 4-Coumaraete CoA ligase-Like5 (*4CLL5*) on the root planlets *Musa acuminata* L. 4-Coumarate CoA ligase-Like5 gene involved in lignin synthesized through 4-Coumarate CoA ligase Like-5 enzyme in phenyl-propanoid pathway.

Genes expression level 4-Coumarate CoA Ligase like-5 in application variative, 4-Coumarate CoA Ligase Like-5 gene is expressed for all application, up regulated in NaCl 0.05 and 0.10 M, down regulated in NaCl 0.15 and 0.20 M.

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