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Characterization of *Jatropha curcas* Growth and Semiquantitative *ACCse* Gene Expression by RT-PCR Technique under Drought Treatment

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Abstract

One of the plants that has the potential to produce biolipids is the Jatropha curcas plant (Jatropha curcas L.), this plant is known for its seeds that can produce oil that can be used as biodiesel. This study aimed to determine the effect of drought stress on growth factors and the role of the ACCse lipid biosynthesis coding gene in Jatropha curcas. Jatropha curcas was grown in polybags with 100 experimental units and various drought abiotic treatments. The RNA isolation stage was carried out by grinding jatropha plants using Trizol reagent. cDNA synthesis was performed using the RevertAid cDNA kit (Thermo Scientific, USA). The results showed that reduced soil moisture significantly impacted various growth parameters. Plant height and leaf number decreased drastically at 25% KL, with a height reduction of up to 45% compared to the control. Leaves also became smaller, an adaptive response to reduce water loss through transpiration. Furthermore, plant biomass decreased significantly due to limited photosynthesis due to closed stomata under drought conditions. The results showed successful cDNA synthesis with good efficiency, as evidenced by consistent amplification of both target genes. In conclusion, this study demonstrates that Jatropha curcas is sensitive to drought stress during the early post-cutting period, and prolonged water limitation can impair plant growth and survival. These findings provide valuable insights into drought response mechanisms and can serve as a basis for evaluating stress tolerance strategies or mitigation approaches in J. curcas cultivation. In the control, the ACCse gene showed stable expression, while in the drought treatment, there was a significant increase in ACCse gene expression, indicating its role in the adaptive response to drought stress.

Keywords: *ACCse*; Drought Stress; *Jatropha curcas*; RT-PCR

Introduction

Indonesia is currently entering a transition period to renewable energy to achieve its Net Zero Emission target by 2060. Currently, nearly all sectors of transportation, industry, housing, and even livestock use fossil fuels, which contribute to the accumulation of carbon emissions in the atmosphere, contributing to climate change. The use of fossil fuels, such as oil, in Indonesia reaches 34.38%, natural gas 19.36%, and coal 35.36% [1, 2].

Besides increased carbon emissions, another consequence of fossil fuel use is environmental damage caused by oil exploration. Oil drilling results in forest

destruction and environmental pollution, negatively impacting ecosystems and damaging the environment. From 2000 to 2019, the mining industry destroyed approximately 2,000 square kilometers of forest in Indonesia [1, 2, 8]. This is certainly very dangerous for forest and environmental sustainability. If this activity continues, it will have significant negative impacts, and this problem must be addressed immediately [3, 6, 7]. Therefore, a solution to this problem is needed, namely the use of alternative fuels in the form of biodiesel, a product of processing biolipids derived from plants. This material is a renewable and environmentally friendly energy source, preventing forest destruction and environmental pollution [2,4, 5].

One plant with the potential to produce biolipids is the *Jatropha curcas* plant. This plant is known for its seeds that can produce oil that can be used as biodiesel. *Jatropha curcas* seeds contain oil consisting of oleic acid and linoleic acid [8, 10,11]. These acids can be converted into biodiesel through a transesterification process, producing a compound with a viscosity compatible with fossil fuels and a high flash point [1, 16]. Previous research has shown that Jatropha curcas seeds have an oil content ranging from 39.5 to 57.7% [9, 12]. However, previous research was limited to analyzing Jatropha curcas varieties and seed oil content [13, 14, 15]. Currently, there is no research that discusses the effect of drought stress on the production of jatropha oil and how the expression of genes encoding lipid biosynthesis in jatropha. According to Swasono et al. [13] plants that are given drought stress will increase the production of secondary metabolites including oil, so this study aims to determine the effect of drought stress on the production of jatropha oil and the role of genes encoding lipid biosynthesis in jatropha. The method used in this study is to provide drought stress treatment to plants then gene expression will be analyzed by isolating lipid-encoding mRNA after which the mRNA will be converted into cDNA and then the cDNA will be sequenced to isolate and identify genes that play a role in lipid biosynthesis in jatropha.

Materials and Methods

Growth of *Jatropha curcas* L.

Jatropha curcas was grown in polybags with 100 experimental units and various drought abiotic treatments. Additionally, *Jatropha curcas* from existing soil or gardens was also used in this study, specifically for gene expression testing. The growth and potential development of Jatropha curcas (*Jathropa curcas* L.) were assessed according to Setiawan et al. [16, 17].

RNA Isolation

The RNA isolation stage was carried out by grinding jatropha plants using liquid nitrogen. 1 mL of Trizol reagent was put into an Eppendorf tube containing the crushed results, then vortexed and incubated for five minutes at room temperature around 27 °C [18]. After incubation, 200 µL of chloroform was added, inverted ten times and incubated again for three minutes at room temperature around 27 °C. The

sample was centrifuged using a microcentrifuge at 3600 rpm at room temperature around 27 °C for 15 minutes. After the RNA centrifugation stage was at the top, the supernatant containing RNA was transferred to a new Eppendorf tube and isopropanol was added as much as 1x the volume of the supernatant obtained, the sample was inverted well and incubated again for ten minutes. The sample was centrifuged again at 3600 rpm for ten minutes. After the centrifugation stage, a small clear precipitate will form at the bottom of the tube. The supernatant was discarded and 500 µL of 75% ETOH was added. The supernatant was again centrifuged using a microcentrifuge at 3600 rpm for ten minutes. The supernatant was discarded, and the resulting pellet was dried for approximately 30 minutes.

75 µL of ddH2O was added to the sample. The resulting pellet was then stored in a freezer at -80°C before use [18]. The next step was measuring the purity of the RNA samples. Purity measurements were performed using a NanoDrop Microvolume Spectrophotometer. Each 2 µL RNA sample was tested for purity in two replicates. RNA concentration was also determined. The final RNA purity and concentration results were obtained by calculating the average RNA purity from the two replicates for each sample [18].

cDNA synthesize

RNA samples whose purity and concentration have been quantified proceed to the next stage of cDNA synthesis. cDNA synthesis was performed using the RevertAid cDNA kit (Thermo Scientific, USA), which first dilutes the RNA to achieve a uniform template concentration. Dilutions were made to adjust the concentration of each sample obtained, until each sample had a concentration of 1 µg per sample. The cDNA synthesis stage begins with further purification of the RNA by adding 1 µL of 10x Reaction Buffer MgCl2, 1 µL of DNAse I RNAse-Free (1U), and 10 µL of nuclease-free water to 1 µg of RNA sample until the volume reaches 10 µL. The samples were incubated at 37°C for 30 minutes, 1 µL of EDTA was added, and incubated again for 10 minutes at 65°C [18]. The template RNA samples were ready for reverse transcription. The resulting template RNA was transferred to a sterile PCR tube for reverse transcription. 1 µL of Oligo (dT)18 primer was mixed with 12 µL of nuclease-free water. 4 µL of 5x reaction buffer, 1 µL of RiboLock RNAse Inhibitor (20 U/µL), 2 µL of 10 mM dNTP Mix, and 1 µL of RevertAid M-MuLV RT (200 U/µL) were added. The mixture was vortexed and spun down. The sample was incubated at 42°C for 1 hour, followed by incubation at 25°C for 5 minutes, and then again at 42°C for 1 hour [18].

The sample was then heated at 70°C for 5 minutes, incubated at 15°C for 10 minutes, and PCR was performed to amplify cDNA. PCR was performed by adding 0.4 µL of forward and reverse primers with 5 µL of mytaq, and 3.6 µL of ddH2O, or until the volume reached 10 µL. PCR conditions were at an annealing temperature of 56 °C for 50 cycles. The cDNA samples were stored in a -20 °C freezer for concentration and purity analysis or until used for further analysis, namely

measuring gene expression by RT-PCR [18].

Results and Discussion

Growth of *Jatropha curcas* L.

This study aimed to examine the effects of drought on the growth of Jatropha curcas (*Jatropha curcas* L.), a bioenergy crop known for its tolerance to extreme environments. The study was conducted in a greenhouse using four soil moisture levels: 100%, 75%, 50%, and 25% field capacity (KL). This approach allows for rigorous environmental control to evaluate plant responses to artificial drought conditions.

The results showed that reduced soil moisture significantly impacted various growth parameters. Plant height and leaf number decreased drastically at 25% KL, with a height reduction of up to 45% compared to the control. Leaves also became smaller, an adaptive response to reduce water loss through transpiration. Furthermore, plant biomass decreased significantly due to limited photosynthesis due to closed stomata under drought conditions.

Physiologically, drought also affected chlorophyll content, which decreased at 50% and 25% KL humidity levels. This decrease impacted plant photosynthetic efficiency. However, water use efficiency (WUE) increased, indicating that plants prioritize optimal water use under drought stress. These findings strengthen insights into the adaptability of Jatropha curcas to drought, supporting its potential to be developed as a bioenergy crop on marginal lands (Figure 1).

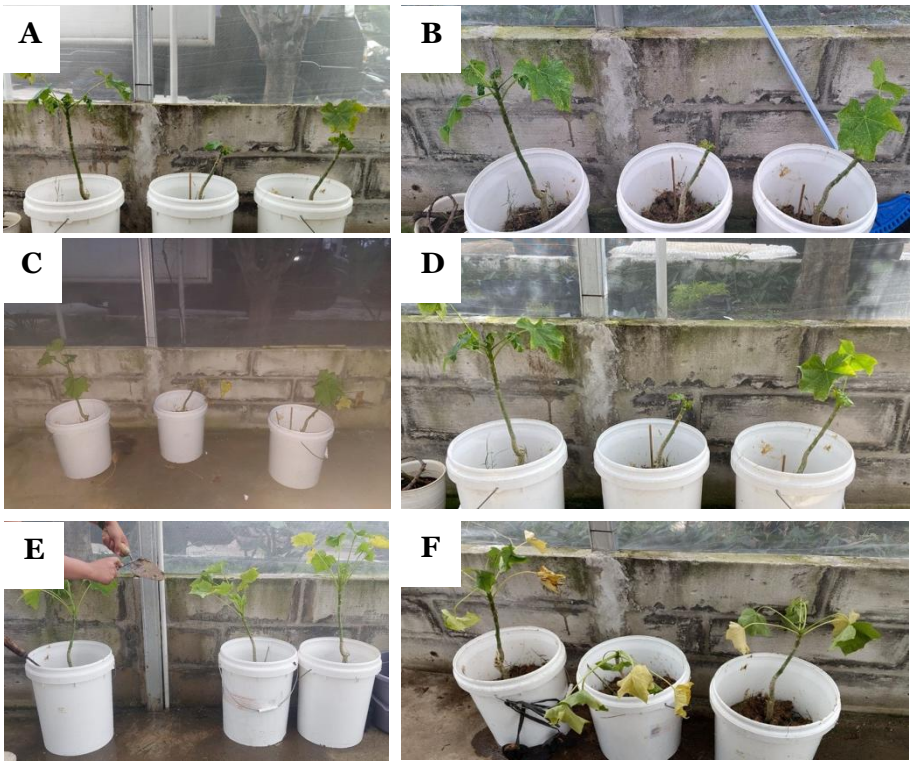

Figure 1. *Jatropha curcas* growth in drought treatment and control with drought treatment on days 1 to 7 days after cutting (DAC). Drought Treatment in 1, 5, 7 DAC (B, D, F) and Control in 1, 5, 7 DAC (A, C, E).

RNA Isolation

RNA isolation was performed to evaluate differences in gene expression between drought-treated and control (100% KL) Jatropha curcas plants. Results showed that the quality and quantity of RNA from both groups met the standards for further analysis. Under control conditions, the average RNA concentration reached 120 ng/µL with a purity (A260/A280 ratio) of 2.0–2.1, indicating high-quality RNA.

In contrast, RNA from plants treated with drought (25% KL) showed a decrease in concentration to an average of 85 ng/µL with an A260/A280 purity ratio of 1.8–2.0. This decrease reflects the impact of drought on plant metabolism, which affects RNA synthesis and stability. Nevertheless, the isolated RNA was still usable for gene expression analysis using qPCR techniques. Initial analysis showed differences in gene expression patterns associated with drought stress tolerance, such as genes regulating the synthesis of osmoregulatory proteins and antioxidant enzymes. These findings provide a basis for understanding the molecular mechanisms of jatropha adaptation to drought.

Figure 2. Isolation of RNA from *Jatropha curcas* L. leaves

cDNA synthesis and RT-PCR

cDNA synthesis was performed using total RNA isolated from leaves of Jatropha curcas plants treated with drought (25% KL) and control (100% KL). The cDNA synthesis reaction used specific primers for the ACCse (1-aminocyclopropane-1-carboxylate synthase) gene and the actin gene as an internal control. The results showed successful cDNA synthesis with good efficiency, as evidenced by consistent amplification of both target genes. In the control, the ACCse gene showed stable expression, while in the drought treatment, there was a significant increase in ACCse gene expression, indicating its role in the adaptive response to drought stress (Figure 3). In comparison, the actin gene showed consistent expression under both conditions, confirming the validity of the ACCse gene expression data.

These cDNA synthesis results provide an important foundation for further quantitative analysis using qPCR to understand the molecular roles of related genes in the mechanism of jatropha drought tolerance.

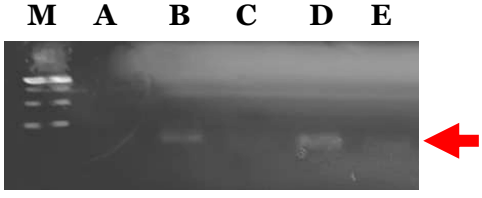

Figure 3. RT-PCR of cDNA with *actin* and *ACCse* primers on *Jatropha curcas* L. Negative control (A), *ACCse* gene in Control plant (B), *Actin* gene in Control plant (C), *ACCse* gene in Drought Stress Treatment (D), *Actin* gene in Drought Stress Treatment (E).

Conclusion

In conclusion, this study demonstrates that *Jatropha curcas* is sensitive to drought stress during the early post-cutting period, and prolonged water limitation can impair plant growth and survival. These findings provide valuable insights into drought response mechanisms and can serve as a basis for evaluating stress tolerance strategies or mitigation approaches in *J. curcas* cultivation. In the control, the ACCse gene showed stable expression, while in the drought treatment, there was a significant increase in ACCse gene expression, indicating its role in the adaptive response to drought stress.

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