The Effect of Light Colour on Rbcl Gene Expression of *In-Vitro* Lemba (*Curculigo latifolia)*

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Abstract. *Curculigo latifolia* is an endemic species of Borneo Island which has great potential as a medicine or artificial sweetener. However, this species only exists in the wild and is not cultivated. Therefore, in-vitro techniques by controlled environment condition such as light can be use to increase plant productivity. Plants absorb light using various types of photoreceptors and influence gene expression to stimulate physiological responses, including photosynthesis. One of the stages of photosynthesis is the Calvin cycle, where the process of CO_2 fixation catalyzed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Rubisco is composed of eight small subunits (SSU) and eight large subunits (LSU). LSU is encoded by *rbcL* and plays an important role in the initiation of carbon fixation. Therefore, this study aims to determine the effect of light type on *rbcL* gene expression in lemba (*Curculigo latifolia*). This study used seedling induced from seed grown on MS0 (Murashige and Skoog) media with light treatment of red LED, blue LED, white LED, and white fluorescence. *rbcL* gene expression were analyzed using Real-Time Quantitative PCR. The results showed that the highest *rbcL* gene expression is detected in seedlings with blue LED light exposure treatment, which is 3.81 fold. While the lowest was found in seedling exposed to white fluorecent which is 0.37 fold. This discovery is expected to provide insight into the best environmental conditions to optimize the productivity of *C. latifolia.*

1. Introduction

Curculigo latifolia or known as Lemba by the people of Kalimantan is a herb that belongs to the Hypoxidaceae family and it is an endemic species of Kalimantan Island. In Indonesia, this plant can be found in Sumatra, Bangka, Papua, and Java. In addition, the plant is also reportedly distributed in West Africa, India, Burma (Myanmar), Thailand, Malaysia (Perak, Pahang, Serawak, and Sabah), and the Philippines (Palawan, Balabac, and Samar) [1]. The entire part of this plant can be useful, where the leaves are used as fishing nets, ropes, and

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materials for weaving. The plant's fruit also contains a sweetening protein called curculin, which can change sour tastes into sweet and it is 500 times sweeter than sugar. The leaves and flowers are also used as medicine for high fever, while the rhizome was use to treat stomachaches and make ointments for wounds. Therefore *C. latifolia* has great potential for low calorie sweetener and medical plant [1]. The high potential for plant utilization is in contrast with the challenging of its propagation. This species is is mostly found in the wild and not cultivated. Its has recalcitrant seeds which have a low germination viability. Therefore, in-vitro plant culture by controlled environment condition such as light to can be use as an alternative method for propagating to increase plant growth and productivity [1].

Light as a signal can influence the plant growth and development. The signals received affect gene expression and can stimulate different physiological and metabolic responses in plants [2]. Plants absorb a type of light called Photosynthetically Active Radiation (PAR) with a wavelength of 400 nm to 700 nm consisting of blue (400 ~ 510nm) and red (610 ~ 720nm) light. Red light signals are received by phytochromes as photoreceptors and facilitate plant responses such as germination, de-etiolation, and shade avoidance responses. As for blue light, it is received by cryptochromes and phototropins and regulates various responses such as, de-etiolation, inhibition of hypocotyl elongation, photoperiodic flowering, guard cell development, stomatal opening, leaf senescence, pathogen response, to optimize photosynthesis ability [3].

Photosynthesis consists of a light reaction which involves light-driven electron and proton transfer, then continues in the dark reaction where CO_2 fixation occurs into carbohydrates through the Calvin-Benson cycle using ATP and NADPH energy produced during the bright reaction. In the carbon fixation process, CO2 is bound with *ribulose 1,5-bisphosphate* (RuBP) and catalyzed by the enzyme *ribulose-1,5-bisphosphate carboxylase/oxygenase* (RuBisCO). RuBisCO is composed of eight small subunits (SSU) encoded by a multigene family (*rbcS*) in the nucleus, and eight large subunits (LSU), encoded by a single gene (*rbcL*) in chloroplasts [4]. Activation of RuBisCO depends on LSUs, where each LSU contains one active side that functions to bind RuBPs that play a role in CO_2 fixation. An increase in RuBisCO synthesis leads to an increase in photosynthetic capacity which leads to an increase in plant biomass production 51]. The carboxylation capacity of RuBisCO can be can be enhanced by modifying the LSU or SSU genes to improve their catalytic properties or by modifying the environment to increase the expression of these genes [6]. Based on the above, the aim of our research was to study the influence of light color on the *rbcL* gene relative expression of *C. latifolia* in vitro.

2. Matrials and methods

2.1 Plant material

The samples used in this experiment are leaves from seedlings propagated in-vitro from seeds cultured on MS0 media according to the research of Muslihatin et al. (2023) [7]. The seedling have been incubated for 20 weeks under different light condition as treatments: blue, red, and white LED light, and also white fluorescent light at 16 h-light /8 h- dark photoperiod**.**

2.2 Primer designing and reference genes

Gene sequence of encoding the large subunit of Rubisco (rbcL) was retrieved from GenBank NCBI (https://www.ncbi.nlm.nih.gov) through the nucleotide search menu. A complete sequence 1296 bp rbcL gene cluster with accession number JX290067.1 was selected in this study. Primer pairs for qRT-PCR (**Table 1**) were designed using the online software

OligoAnalyzer (https://sg.idtdna.com/calc/analyzer). The selection of primers was guided by specific criteria, including an optimal length of 20–25 nucleotides, melting temperature ranging from 60-65°C, GC content < 50%, product size range 100-446 base pairs, the absence of self-complementary sequences at the 3' end, and absence of hairpin structures and self-dimers. The reference gene used in this study is the Ubiquitin gene with primers according to [8].

Table 1. qRT-PCR Primer pairs.

Primer name	Sequence (primer direction 5'-3')
rbcL-Fw	CGT TGG AGA GAC CGT TTC TTA T
rbcL-Rv	TCT TCA CAT ATA CCC GCA GTT G
Ubiquitin -Fw*	TAT AAT CTG CAA GGG TCC GGC
Ubiquitin -Rv*	AGA TTC AGG ACA AGG AGG GG

*Note : according to [8]

2.3 mRNA isolation and cDNA synthesis

RNA was isolated from 50mg of seedling leaves in each treatment using the Geneid Total RNA Mini Kit (Plant) which steps including lysis, RNA binding, purification, and RNA elution. Quantification of total RNA concentration was performed using Nano Drop (Thermo Scientific™ NanoDrop 2000) The wavelength used was A260/A280. The ideal RNA purity obtained from the RNA isolation process is 95-100% with an absorbance of 1.8-2.0.

cDNA was synthesized from total RNA using GoScriptTM Reverse Transcription System (Promega, USA) according to the manufacturer's instructions. The reaction was placed in a heat block and incubated at 25°C for 5 min for annealing and 42°C for 60 min for extension. This was followed by incubation at 70°C for 15 minutes to inactivate reverse transcription.

2.4 Real-Time qRT-PCR

Analysis of *rbcL* gene expression was performed by Real-Time qRT-PCR method using *GoTaq® qPCR Master Mix* (Promega, USA) with 12.5 µL GoTaq® qPCR Master Mix, 5.5 µL Nuclease Free Water, 1 µL forward primer, 1 µL reverse primer, and 5 µL cDNA. The primers used can be seen in **Table 1**. This analysis was carried out using a Real-Time PCR machine (MyGopro, UK) at 95°C for 120 seconds, and 40 cycles for 15 seconds at 95°C, and 60 seconds at 60°C. qRT- PCR in each treatment was run in three replicates with the Ubiquitin gene as the reference gene. The qRT-PCR result data in the form of Threshold Cycle values.

2.5 Data analysis

The relative changes in gene expression from real-time quantitative PCR experiment generates CT (Cycle Threshold) Values. Then, the CT values were calculated using the $2^{-\Delta\Delta CT}$ method by Livak & Schmittgen (2001) using Microsoft Excel 365. Statistical analysis was performed using SPSS 22 for one-way analysis of variance (ANOVA) followed by a Tukey test at the significance level of 0.05 [16]. All experiments consisted of three replication.

3. Results and Discussion

To examine the effect of light color on *rbcL* gene expression, in vitro *C. latifolia* seedlings were exposed to different light colors for 20 days. RNA samples were obtained by extracting leaves from sterile seedlings under 4 light treatments as shown in **Fig. 1**. Changes of *rbcL* gene expression levels were detected using Real-Time qRT-PCR with SYBR® Green florescence dye, which binds to DNA fragments during amplification and can be used to quantify amplicons. The house keeping gene used in this study was Ubiquitin gene.

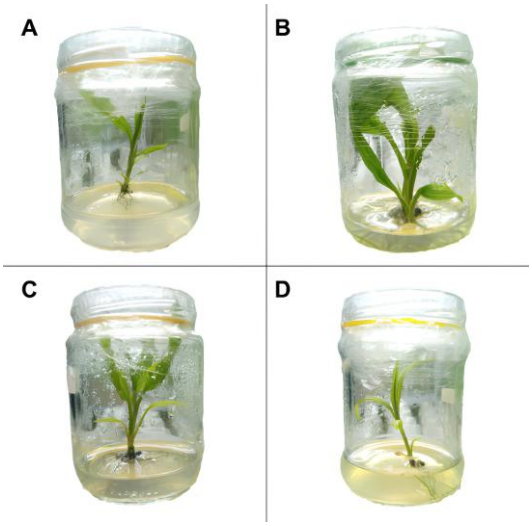

Fig. 1 Seedling of *Curculigo latifolia* on different light condition (A) White Fluoresens (B) White LED (C) Blue LED (D) Red LED.

The results of statistical analysis using oneway ANOVA showed that light color treatment affects the expression of the *rbcL* gene in *Curculigo latifolia* p value = 0.018 ($p < 0.05$). As shown in **Fig. 2**, the relative gene expression of *rbcL* gene varied with different light color. The results indicate the fluctuating expression of the *rbcL* gene of both upregulation or downregulation.

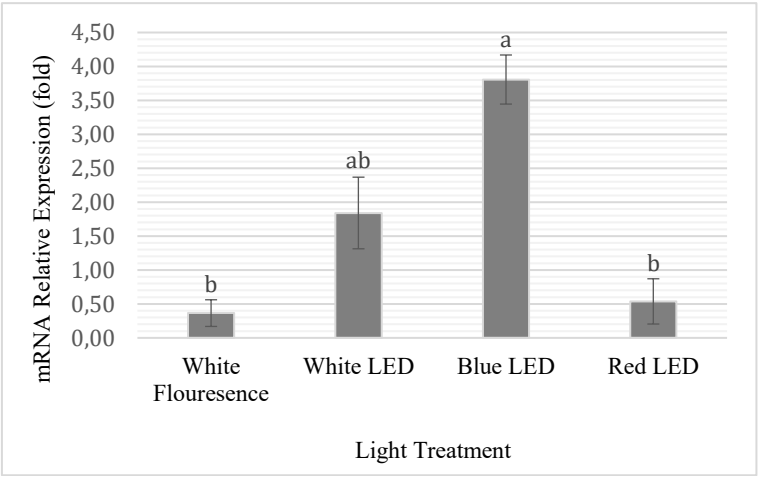

Fig 2. Effects of light color on relative mRNA expression of the *rbcL* genes.

The lowest gene expression was found in seedling exposed to white fluorescent which is 0.37 fold.. While the relative gene expression values of *rbcL* in white, blue and red LED light treatments were 1.84 fold, 3.81 fold, and 0.54 fold, respectively. The highest relative value of mRNA gene expression is found in planlets with blue LED light exposure treatment with significant differences compared to other treatments. Based on the lamp type, LED have a higher relative gene expression of *rbcL* compared to the treatment with fluorescent lamp.

Curculigo latifolia is a monocotyledonous plant classified as an annual herb, clumped with a height of less than 2 m. This plant has complete leaves, ensiform, parallel venation, petioles with a length up to 1/3 of the total leaf length, pseudo-stem consisting of overlapping leaves. Inflorescence arising directly from rhizome (Radical inflorescens), yellow trimerous flowers, with 6 petals, 6 stamens and an inferior ovary. The fruit is berry-type, black in color with a sweet taste. There are many seeds found in a fruit which has hard and thick coat. Lemba is found in highland areas with altitudes between 1500-2000 masl. and generally grows on slopes and forest areas [1,9,10].

Environmental signals, such as light play an important role in initiating gene expression during plant development including photosynthesis proces [11]. In the present study, seedlings grown under monochromatic (Blue and Red) LED and white fluorecest, as compared with white LED, show a significantly affect on the photosynthesis-related gene *rbcL*. The relative gene expression of rbcL was increased under blue light LED treatment, but it was decreased under red LED and white Fluorescent.. This related to previous research reported by Su et al. (2014), which showed 150% increase in *rbcL* transcription under blue light exposure in *Cucumis sativus* L. Another study on *Actinidia chinensis* also showed an increase in *rbcL* gene expression in line with an increase in the intensity of blue light treatment. While plants exposed to red monochromatic light showed the lowest expression [12].

The increase in *rbcL* gene expression on blue light treatment is related to the synthesis of sigma factor (SIG), a transcription initiation factors in the plastid genome. SIG is a component of the *plastid-encoded RNA polymerase* (PEP) complex that plays an important role to recognize and bind to specific promoter sequences in DNA, marking the starting point for transcription [13]. SIG are encoded in the nuclear genome and translocated into the plastid. In the process of RuBisCO large subunit (LSU) synthesis, the sigma factors that recognize the *rbcL* promoter are SIG1 and SIG5. Basically, transcription and translation of all types of SIGs are affected by the presence of light. However, the most effective wavelength for the synthesis of these sigma factors is the blue light (400 ~ 510nm) [13]. This is because SIG gene transcription is mediated by cryptochrome, which is a blue light photoreceptor [13]. The downregulated of *rbcL* gene expression in red light treatment as a result of cryptochrome photoreceptors that are unable to detect blue light which inhibits the expression of SIG5 and SIG1 genes. Resulting in inhibition of PEP assembly and leading to a decrease in rbcL gene expression in the plastid. Another study showed that the phytochrome mediated response to red light exposure and also the transcription of SIG1 but with a much lower than blue light [14]. The signal transmission pathway begins with photon energy from blue light detected by the Flavin Adenine Dinucleotide (FAD) chromophore on the cryptochrome photoreceptor (CRY). Uptake of photon energy then leads to activation of CRY through phosphorylation and structural changes. CRY interacts with a series of transcription factors and induces SIG5 and SIG1 gene expression [13,14]. Sigma factor then translocated into the chloroplast. In the chloroplast, the assembly of plastid-encoded RNA polymerase (PEP) complex occurs, which combines SIG with PEP subunits (α2, β, β', β") [13]. In the end, initiation of *rbcL* transcription begins with SIG recognizing and binding to promoter elements -10 and -35.

Depending on the light source, seedling exposed to fluorescent lights have lower gene expression levels than LED lights. This is because fluorescent lamps and LED lamps transmit light in different spectra. Fluorescent lamps produce light by passing an electric current through a gas, which causes the gas to release ultraviolet (UV) light. The UV light is then converted into visible light by the phosphor coating on the inside of the lamp. While LED lights emit light through electroluminescence in semiconductor materials, and transmit light in a limited spectrum depending on the properties of the semiconductor [15]. In this study, downregulated *rbcL* gene expression under fluorescent light treatment is a response to high light intensity. Fluorescent lamps emit light over a broader range of the spectrum including a large amount of UV light. It causes a downregulation of *rbcL* expression as a protective mechanism to prevent damage to the photosynthetic machinery.

4. Conclusion

Light is one of the important factors in plant growth. Each spectrum of light affects gene expression and can stimulate different physiological and metabolic responses in plants, including photosynthesis. However, to increase the rate of photosynthesis, exposing plants using artificial light with a specific spectrum will be a very important tool to increase plants productivity. In the present study, in-vitro seedlings of *C.latifolia* grown under monochromatic light including white, red and blue LED, as well as white fluorecent. The research focuses on the influence of light color on the relative expression of the *rbcL* gene, a key gene involved in the calvin cycle of photosynthesis. The results indicated that the highest relative gene expression of *rbcL* is found in seedling under blue LED light treatment. This occurs due to the encoding of transcription initiation factors that play a role in the transcription process of the rbcL gene induced by blue light.

The authors gratefully acknowledge the financial support from Institut Teknologi Sepuluh Nopember for this work under project scheme of the publication writing and IPR incentive program 2025

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