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Research Article

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Expression of the curculin gene from different organs of *Curculigo latifolia* under different culture conditions

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ABSTRACT

Curculigo latifolia is the main source of curculin. Curculin is a special kind of protein that tastes sweet and can change the way food tastes, and is naturally expressed in fruit. This study aims to measure and compare the relative expression of the curculin gene in different organs, i.e., fruit and leaf, under in vitro and in vivo conditions. In this study, mRNA isolation was carried out in tissues derived from fruits and leaves grown in vitro and in vivo (in the soil) of *C. latifolia* from West Java, Indonesia. Leaves from 20 weeks seedling on polybag, fruit 40 days after anthesis, and leaves from 20 weeks seedling of *C. latifolia* cultured on MS0 medium that were used in this experiment. The relative expression was measured using qRT-PCR. The results showed that the comparison of curculigo's parts in the leaves was lower than in the fruit. The lowest expression was obtained in leaves grown under in vitro conditions at 0.001-fold, while leaves grown in the soil at 0.566-fold compared to curculin gene expression in fruit. This study concludes that the expression of the Curculin gene in fruit is different between the fruit and leaves, and the growth conditions have an influence on Curculin gene expression, where Curculin gene expression under in vitro conditions is lower than expression under in vivo conditions. The curculin is not only expressed in the fruit but also the leaves, so it has the potential to be developed.

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INTRODUCTION

Curculin is a special kind of protein that tastes sweet and can change the way food tastes. Indonesian-grown *Curculigo latifolia* is the source of curculin. Apart from its ability to alter taste, curculin

also has a sweet taste. First, Yamashita et al. isolated curculin. It was extracted from Curculigo latifolia fruits using 0.5 M NaCl, and it was then purified using gel filtration, CM-Sepharose ion-exchange chromatography, and ammonium sulfate fractionation (Yamashita et al., 1990; Yamashita et al., 1995). Thus obtained, pure curculin provided. It has been demonstrated that curculin is 500–9,000 times sweeter by weight and potential than sucrose. for used as a low-calories sweetener (Nookaraju et al., 2010; Yamashita et al., 1990; Abe et al., 1992; Suzuki et al., 2004). When sugar intake is high, particularly when sugar content is high in meals, there can be changes in food intake. This shifting trend has also led to a widespread use of artificial sweeteners by food processing industries. The rising use of high-calorie, high-energy meals, especially sugar and other fructose-sweetened goods, raised worries about the rising incidence of metabolic illnesses such as type II diabetes, obesity, and metabolic syndrome (Anton et al., 2010; Kristanto & Hartono, 2021). With 463 million cases globally, diabetes mellitus is still a major global health issue. In terms of the global prevalence of type 2 diabetes patients, Indonesia has a high number of sufferers and is ranked fifth (Bistara et al., 2022; Rohmawati et al., 2023; Sari et al., 2023). A strategy to decrease the utilization of artificial sweeteners is to employ naturally sweeteners with low or zero calories, such as Curculin from *C. latifolia*.

Curculigo latifolia is the main source of curculin only occurs naturally in the wild and cannot yet be domesticated (Raden et al., 2017). The plant develops slowly, which makes standard propagation challenging. It demonstrated that seeds could not sprout. It is believed that the seeds need a unique medium and attention because they are resistant. In vitro propagation might be a viable substitute. In vitro culture of *C. latifolia* was conducted by many researchers (Babaei et al., 2013; Babaei et la., 2014; Farzinebrahimi eta al., 2016; Umar et al., 2023; Muslihatin et al., 2024).

Scientists have been attempting to comprehend the morphological, physiological, biochemical, and molecular modifications connected to tissue culture responses since the development of plant cell and tissue culture procedures. Gene activity is regulated at several levels in plants and other eukaryotes, with transcriptional control being the main and best-understood method of regulating gene expression (Riechmann, 2002). Gene expression is limited to specific tissues, developmental stages, or growth conditions to produce a confined pattern (Yaschenko et al., 2022). Furthermore, each plant tissue or organ possesses unique features due to genes particular to that tissue or organ. Tissue/organ-specific differences in metabolites and gene expression have recently been reported in olive fruit, young leaves, and old leaves (Xiaoxia et al., 2020).

Although Cur mRNA and cDNA clones for curculin have been isolated and sequenced, expression of the Curculin gene encoding Curculin of *C. latifolia* under different conditions and in specific organs has not been reported yet (Abe et al., 1992; Suzuki et al., 2004). It will be beneficial to do a study on the specificity of organ expression and patterns of expression in response to signals from the environment and development. Studying gene expression is crucial since it controls the amount of proteins that a given cell must produce by acting as an "on" and "off" switch. The cell will create more if more is needed; otherwise, it will produce less. This study aims to measure and compare the relative expression of the curculin gene in different organs, i.e., fruit and leaf, under in vitro and in vivo conditions.

RESEARCH METHODS

Leaves from 20 weeks seedling on polybag, fruit 40 days after anthesis, and leaves from 20 weeks seedling of *C. latifolia* cultured on MS0 medium according to Muslihatin et al. (2023) were used in this experiment. The complete complementary DNA (cDNA) of the Curculin genes (Accession: AB181490.1) was sourced from the NCBI public platform and utilized for querying. OligoAnalyzer, an online program,

was used to construct primer pairs for curculin genes (https://sg.idtdna.com/calc/analyzer). The ideal primer length was 20–25 nucleotides, the melting point was 60–65°C, the GC content was less than 50%, the product size range was 100–446 base pairs, there were no self-complementarities at the 3' end, and there were no hairpin structures or self-dimers. The same procedures were used to design the qRT-PCR primers (which were provided in Table 1). Ubiquitin was the reference gene used in this investigation. The primer utilized for the Ubiquitin gene, as reported by Okubo et al. (2021).

The 50 mg of fruits and leaves were ground up. As directed by the manufacturer, total RNA was isolated using the Total RNATM Mini Kit (Plant Geneaid). Using the Nanodrop (Thermo ScientificTM Nanodrop 2000), RNA quality was further evaluated. The GoScriptTM Reverse Transcription System (Promega, USA) was used to create the cDNA from total RNA in accordance with the manufacturer's instructions. The reactions were incubated for five minutes at 25°C in a controlled-temperature heat block before being incubated for sixty minutes at 42°C. It is possible to optimize the extension temperature for fifteen minutes at 70°C. 3 µL of the cDNA sample was mixed with 25 µL NEXproTM HS PCR 2X Master Mix, 5 µL *Nuclease Free Water*, 1 µL Primer Forward, 1 µL Primer Reverse (Primers are listed in Table 1). The reactions were placed in a thermal cycler (Select cycler II, Taiwan). This study used 35 cycles with preheated to 95°C for 7 minutes, denaturing at 95°C for 30 seconds, temperature of annealing at 54°C for 30 seconds, temperature of extension at 72°C for 40 seconds, and final extension at 72°C for 7 minutes. Products PCR of cDNA were analyzed or detected using agarose gel electrophoresis.

DNA visualization was performed using the agarose gel electrophoresis technique. Preparation of 2% agarose gel was carried out by dissolving 2 g of agarose in 100 ml of 1X Tris Borate EDTA (TBE) solution and then heating in the microwave for 2 minutes. 20 µL/20 ml of gel dye RedSafeTM (IntRON Biotechnology) was added to the agarose solution (IntRON Biotechnology) and then left for 15 minutes until the solid agarose became a gel. Then the agarose gel was placed in an electrophoresis bath, which contained 1x TBE solution until the gel was submerged. 5 µl of the DNA sample was tested, and 3 µL DNA leader 100 bp was added. Furthermore, the electrophoresis was carried out for 30 minutes at 100V. The concentration and quality of electrophoretic RNA results were observed under a UV transilluminator. The quality of the DNA is shown with a white line.

With a final volume of 25 ml, real-time PCR was carried out in a real-time PCR machine (MyGo pro, UK) under pre-set conditions (95°C for 120 seconds, 40 cycles of 15 s at 95°C for 15 seconds to 60°C, 1 minute to 72°C). GoTaq(R)qPCRMaster Mix (Promega, USA) was used in the reactions. Its ingredients were 1 µL each of the primers forward and reverse, 5 µL of cDNA, and 12,5 µL of GoTaq (R) qPCR Master Mix. The endogenous reference gene for all assays was ubiquitin, and each reaction was conducted three times. The relative changes of genes were analyzed using the 2-ΔΔCT technique (Livak & Schmittgen, 2001). The relative expression of curculin under different growth conditions was analysed by Analysis of Variant (ANOVA) one way, and was continued by Tukey test.

FINDING AND DISCUSSION

In this study evaluated the expression of the curculin gene in different organs and different environmental conditions, in vitro and in vivo can is shown in Figure 1. The primers used in the isolation of the Curculin gene fragment meet the requirements primary design parameters according to Russel & Sambrook (2001) and Borah (2011). The primer used must have a length between 18-28 bases, with a Tm range of 50-65°C and GC levels of 40-60%. Pairs of Curculin forward and reverse primers produce amplicons of 446 bp for the curculin-specific gene and 105 bp for qRT PCR analysis. PCR products were

visualised by electrophoresis (Figure 2). From the visualization using agarose gel. The results showed the visualization (band) of the curculin gene in fruit, leaves under in vivo and in vitro conditions.

Figure 1. Material for mRNA Extraction. (A). Leaves from in Vivo Conditions; (B). Leaves from in Vitro conditions; and (C). Fruit of *C. Latifolia*

Table 1. Primer List for PCR and qRT-PCR Amplification

Nama primer	Primer (5' - 3')	Long	GC%	Tm (°C)	Size
PCR					
curculin fw	GCCAAGTTTCTTCTCACCATTC	22	45.46	61.79	446
curculin Rv	TCCTCATGTTGTGGTTCAGTAG	22	45.46	61.84	
qRT PCR					
curculin fw	GAGTGACGGGAACCTCATTATC	22	50.00	62	105
curculin Rv	CCATCCTGCTGAAGAACAAGA	21	47.60	62.15	
ubiquitin Fw*	TATAATCTGCAAGGGTCCGGC				
ubiquitin Rv*	AGATTCAGGACAAGGAGGGG				

Note: * According to Okubo et al. (2021)

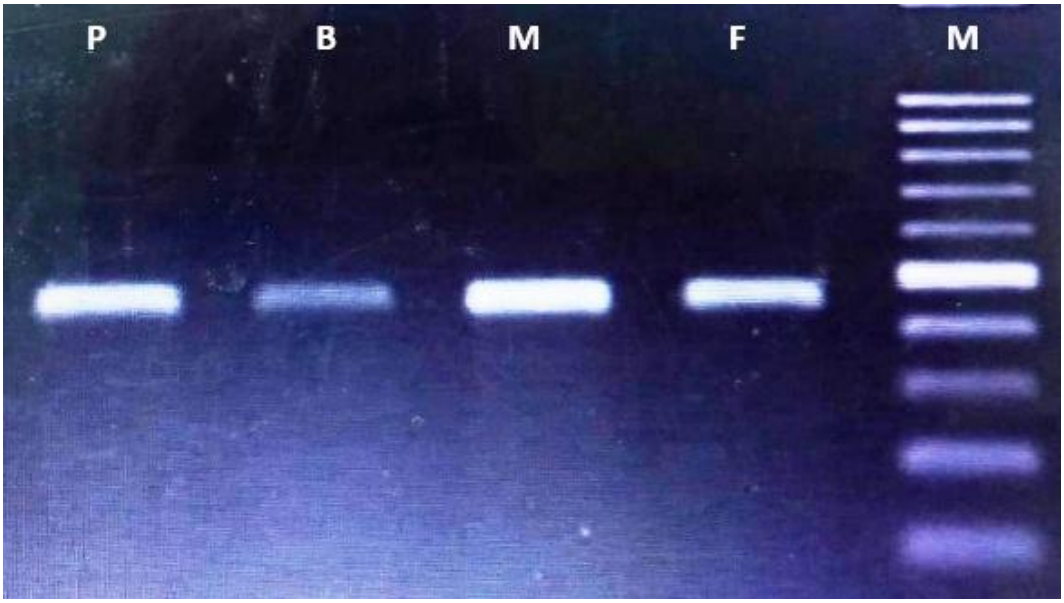

Figure 2. Confirmation of Curculin Gene-Specific PCR Amplification, M: Marker, L: leaf on in vivo, C: fruit; V: Leaf on in Vitro

With its excellent sensitivity and relative simplicity, qRT-PCR is quickly becoming the accepted standard technique for Curculin gene expression investigation. In this study reported that the relative expression of the Curculin gene from leaves is lower than fruit. The lowest relative expression was

obtained on the leaf in in vitro conditions (0.001 fold), and this result is lower than the gene expression of the leaf on in vivo conditions (0.0566 fold), can be seen in Figure 3.

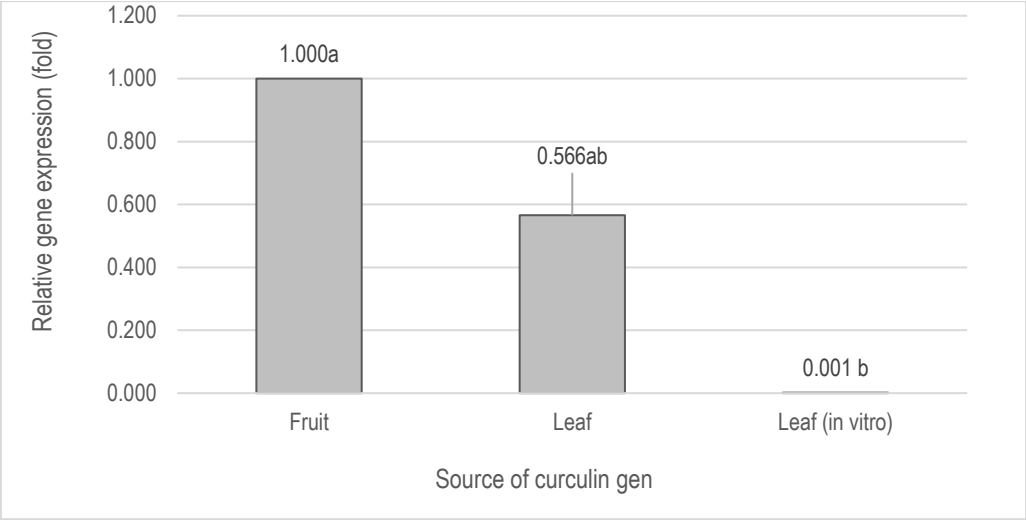

Figure 3. Relative Expression of Curculin Gene

Naturally, the Curculin gene is expressed in fruit, so after eating *Curculigo* fruit, it will taste sweet. The relative expression of the Curculin gene in fruit was significantly different compared to leaves grown in vitro. These results showed that growth conditions influenced the expression of the Curculin gene (sig. < 0.05), in addition, the expression of the Curculin gene was different in each organ. Studies on organ specificity in gene expression have been conducted in many species. Many studies have reported that the genes studied are less expressed in leaves compared to other organs, such as roots, shoots, and flowers (Bazin et al., 2013), but there are studies to the contrary, such as the GRF gene is expressed more in immature Chinese cabbage leaves compared to other tissues (Wang & Ning, 2019). Gene expression research in different organs has been conducted by Yang et al. (2022) who reported GRF gene expression in specific organs of the orchid *Cymbidium ensifolium,* while Xiaoxia et al. (2020) and Ramírez-Tejero et al. (2020) have documented gene expression in different organs in olive trees (*Olea europaea* L.).

In addition, the condition of culture affects gene expression of the curculin gene. Curculin genes were isolated from leaves under different conditions showed significantly different expression. The genetic and epigenetic program of intact plant tissue is thought to be disrupted by in vitro culture, which can result in methylation alterations, transposon activation, chromosomal and DNA sequence differences, and the creation of somaclonal variants. Fitness and adaptability of cultured explant tissue to in vitro cultures ultimately depend on its capacity to reset its genetic and epigenetic program to survive the artificial hormonal environment. These dynamic processes are coordinated at the molecular level. the process's molecular alterations and in vitro cultivation. It is thought that wounding, physical and/or chemical stimuli, the presence of hormones and/or enzymes, in addition to the developmental processes of dedifferentiation and regeneration, cause genomic stress in cultured plant cells. These modifications show up at the level of gene expression.

Genetic alterations such as chromosomal modifications, DNA sequence alterations, amplifications, and transpositions are also linked to in vitro culture. According to McClintock's initial theory, these alterations are thought to be a direct result of cellular stress responses and genome evolution (McClintock, 1984). While it is expected that plants regenerated from these systems will be homogenous, we now know

that there is a high probability of epigenomic and genomic changes, primarily methylation changes, single base pair changes, and small indels, due to intrinsic and extrinsic factors affecting development under artificial conditions. These changes may or may not be associated with phenotypic changes, so all practical applications of these technologies should consider this. It is now understood that endogenous hormonal gradients, and the ensuing gene expression and development they cause, are significantly influenced by the kind, quantity, and timing of exogenous growth regulators introduced to the media (Neelakandan & Wang, 2012).

CONCLUSION

Curculin genes are found in the fruit and leaves of *C. latifolia*. The expression of the Curculin gene in fruit is the highest among the leaves with different culture conditions, and the growth conditions influence Curculin gene expression, where Curculin gene expression under in vitro conditions is lower than expression under in vivo conditions. Although naturally the curculin is expressed in the fruit in this research showed that the curculin was expressed in the leaves, so curculin has potential to be developed.

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