Comparison of \( Cm \) ACS-7 Gene Expression in Melon (\( Cucumis melo \) L.) Flowers

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Abstract. Melon (\( Cucumis melo \) L.) is a member of the Cucurbitaceae family spread throughout the world. Melons have very varied sexual types, which can be important information in genetic analysis and breeding. Sex type jumps from genetic factors, environmental factors, and environmental conditions. The aim of this study was the expression of \( CmACS-7 \) gene expression in melon flowers in various sexual types. This study used melons with andromonoecious sexual types (Melodi Gama 1 and Melodi Gama 3 cultivars) and monoecious (PI 371795 and Bartek). This study uses quantitative PCR analysis using real-time PCR with relative quantitative methods. RNA was isolated and then synthesized into cDNA, then amplified using specific primers. The number of cDNA specific genes was analyzed using Real-time PCR to calculate the level of gene expression. The results showed that the \( CmACS-7 \) gene was expressed more in female and hermaphrodite flowers than in male flowers. In male flowers with andromonoecious sexual type, the \( CmACS-7 \) gene is expressed more than in male flowers with monoecious sexual type. This research is expected to be important information in breeding melon plants.

1. Introduction
Melon (\( Cucumis melo \) L.) is a member of the Cucurbitaceae family found almost all over the world [1]. Melons originated from East Africa [2] and then spread across the Mediterranean region, Asia, America, and even Indonesia [1]. Melons have been cultivated in China around 3000 BC, India around 2000 BC, and Egypt around 1500 BC [3,4]. Prior to 1980, melons were known in Indonesia as imported fruit. Many agribusiness companies have tried to cultivate different varieties of melons from America, Taiwan, China, France, Denmark, the Netherlands, and Germany. Around 1990, numerous varieties of melons had already been developed in Indonesia [5].

Melons have a variety of sexual types that can serve as an important source of information in genetic analysis and plant breeding [6]. Several species of the Cucurbitaceae, including melons, undergo sexual type polymorphisms [7]. Sexual type in melons is influenced by the combination of male, female, and hermaphrodite flowers in a single plant. Sexual types of melons include monoecious (male and female flowers), andromonoecious (male and hermaphrodite flowers), hermaphrodite (hermaphrodite only) and gynoecious (female flowers only) [2,8].
Determination of sexual type in melons is influenced by genetic factors, hormone production, and the environment in which it grows. Ethylene hormone production in melons correlates with pistil formation in flowers, where ethylene inhibitor biosynthesis suppresses female flower growth and induces male flower growth [9]. The formation of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) is the main step in the formation of ethylene [10]. The CmACS-7 is an ACS-gene code which is homologous to the ACS-7 gene in Arabidopsis [7]. This study aims to analyze the expression of CmACS-7 gene in various flowers and sexual types of melon plants.

2. Materials and Methods

2.1. Sample preparation
Samples were collected during the generative phase when male and female flowers appeared on melon plants. The melon samples used in this study were developed at the Laboratory of Genetics of the Faculty of Biology at Gadjah Mada University (Melodi Gama 1 and Melodi Gama 3 cultivars) with andromonoecious sexual types and wild type melons (PI 371795 and Bartek) with monoecious sexual types. The Bartek melon variety is a local variety from Pemalang, Central Java [11]. The flowers were male, female, and hermaphrodite.

2.2. RNA isolation & PCR amplification
RNA was isolated from male and female flowers in plants with sexually andromonoecious types, whereas for the monoecious sexual type, RNA was isolated from male and female flowers. RNA was isolated using the Genejet Fermentas Thermo Scientific isolation kit. The RNA obtained was then synthesized into cDNA (complementary DNA). Amplification of the CmACS-7 gene was carried out using two-step Reverse Transcriptase PCR (RT-PCR). The first stage is cDNA synthesis using the Thermo Scientific Reverse Aid First Strand cDNA Synthesis kit which contains the reverse transcriptase enzyme. The second stage was carried out by amplifying the cDNA that had been synthesized using specific primers [7] (Table 1).

| Primer          | Sequence                                    | Fragment Length |
|-----------------|---------------------------------------------|-----------------|
| qPCR-A_F3       | 5’-TTTAGCCGTCCATGTTGTCGAAACC-3’             | 81 bp           |
| qPCR-A_R3       | 5’-TCATACCGTTCCTTTGAGCCTGTC-3’              | 81 bp           |
| CmActin2_F      | 5’-ATTCTTGGACTCTCTAGTACCTTTCC-3’            | 161 bp          |
| CmActin2_R      | 5’-CCAACCTAAGGGAATAAATCTACC-3’              | 161 bp          |

2.3. Analysis of CmACS-7 gene expression using quantitative PCR (qPCR)
CmACS-7 gene expression was tested using the β-actin housekeeping gene. qPCR was carried out in compliance with the SsoFast™ EvaGreen® Supermix (Bio-Rad) protocol. The primers used for qPCR were the ones used for RT-PCR. The qPCR program consisted of a preliminary 50°C stage for 2 minutes followed by 95°C stage for 10 minutes. The qPCR cycle was carried out 40 times at 95°C for 15 seconds and at 60°C for 1 minute. The qPCR results were then analyzed using the CFX Manager™ version 3.1 software. Gene expression was analyzed using relative quantitation with the Cq method/ΔΔCq method using a housekeeping gene in the form of β-actin. The results of gene expression for each flower type and sexual type were then compared with each other.

3. Result and Discussion

3.1. Result
The amplification results of the CmACS-7 gene and β-actin gene in the melon sample are presented in Figure 1. Figure 1 shows that the DNA band amplified by the CmACS-7 gene has a length of 81 bp,
while the $\beta$-actin gene has a length of 161 bp (Fig. 1). The $\beta$-actin gene was chosen as a housekeeping gene because its expression is not influenced by the environment so it is often used to normalize the quantification of expression. [12–14]. The appearance of DNA bands shows positive result for the presence of the CmA$\text{CS}$-$7$ gene so that it could continue to the qPCR stage to determine the level of gene expression in each flower.

![Image](image_url)

**Figure 1.** The amplification results of the andromonoecious gene (9-16) with a length of 81 bp and actin gene (1-8) with a length of 161 bp; cDNA samples of MG3 hermaphrodite flowers (1,9), MG1 hermaphrodite flowers (2,10), PI 371795 female flowers (3,11), Bartek female flowers (4,12), MG3 male flowers (5,13), MG1 male flowers (6,14), PI male flowers 371795 (7,15), and Bartek male flowers (8,16)

The qPCR result through the cDNA application chart of the sample with a specific primer shows an exponential phase. The linear phase is characterized by a decrease in the chart. Meanwhile, the plateau phase can be seen in some samples. The reaction to the qPCR results is monitored in an exponential phase, so that the value of Cq (quantification cycle) can be identified and the relative value of quantitation can be calculated using the CFX Manager TM version 3.1 software. The qPCR results in the form of an amplification chart are presented in Figure 2.
The qPCR analysis can be carried out with absolute quantification and relative quantification. This study uses analysis in relative quantification. Figure 2 shows that the graph of the amplification results varies with each sample. The highest Cq value was found in the PI 371795 female flower and the lowest was in the control gene. The value of Cq and the relative quantification of the qPCR results are presented in Table 2.

**Table 2. Cq (quantification cycle) values and the relative quantity of qPCR results**

| Target Gene      | Sample             | Cq    | Relative Quantity | E        |
|------------------|--------------------|-------|-------------------|----------|
| Andromonoecious  | MG 3 Hermaprodit   | 23.90 | 3985.86730        | 2.000078585 |
| Andromonoecious  | MG 3 male          | 25.11 | 1714.98838        | 1.999224224 |
| Andromonoecious  | MG 1 Hermaprodit   | 23.63 | 4779.09811        | 1.99915261 |
| Andromonoecious  | MG 1 male          | 24.12 | 3416.04615        | 1.999777042 |
| Andromonoecious  | PI female          | 23.48 | 5298.49573        | 1.999033536 |
| Andromonoecious  | PI male            | 26.59 | 614.16813         | 1.999477199 |
| Andromonoecious  | Bartek female      | 24.00 | 3705.59372        | 1.999877199 |
| Andromonoecious  | Bartek male        | 27.63 | 300.19468         | 1.999958664 |
| Andromonoecious  | Control            | 35.86 | 1.00000           |          |
| Actin            | Housekeeping gene  | 38.82 | 1.00000           |          |

The relative quantity value is obtained from the relative quantity formula of \( E = \frac{\text{Cq}_{\text{sample}} - \text{Cq}_{\text{control}}}{\text{Cq}_{\text{sample}}} \). To obtain the desired value, calculations are carried out using the existing housekeeping gene and calibrator. The results of calculating the relative quantity for various analysis methods are presented in Table 3.

**Table 3. The results of relative quantity calculation in various analysis methods**

| Target Gene      | Sample             | Cq    | Relative Quantity | Livak 2^\Delta\text{Cq} | Pfaffl 2^\Delta\text{Cq} | \( \Delta\text{Cq} \) |
|------------------|--------------------|-------|-------------------|--------------------------|--------------------------|---------------------|
| Andromonoecious  | MG 3 Hermaprodit   | 23.90 | 3985.86730        | 3983.994665              | 3985.8673               | 16.45599            |
| Andromonoecious  | MG 3 male          | 25.11 | 1714.9883         | 1722.155858              | 1714.98838              | 12.3956             |
Andromonoecious MG 1 Hermaprodit 23.63 4779.0981 4803.9319 4779.0981 17.5300
Andromonoecious MG 1 male 24.12 3416.0461 3420.5201 3416.0461 15.6296
Andromonoecious PI female 23.48 5298.4957 5330.2962 5298.4957 18.1567
Andromonoecious PI male 26.59 614.1681 617.3736 614.1681 8.7650
Andromonoecious Bartek female 24.00 3705.5937 3717.1984 3705.5937 16.0751
Andromonoecious Bartek male 27.63 300.1946 300.2457 300.1946 6.8704
Andromonoecious Control 35.86 1.0000 1 1 1
Actin Housekeeping gene 38.82 1.0000

The relative quantity values in Table 3 show that the CmACS-7 gene expression of relative to that of the control and housekeeping gene. Table 3 shows that the CmACS-7 gene expression in female or hermaphrodite flowers was higher than that of male flowers.

3.2. Discussion
The amplification results that looked positive in all flower samples show that the CmACS-7 gene was expressed in all types of melon flowers, but with different levels of expression for each type of flower. This is in accordance with statement [15] that the expression of sexual types in Cucumis melo correlates with ethylene production. Ethylene is related to many aspects of the plant life cycle, including seed germination, root growth, aging of flowers, abscission and fruit ripening [16,17].

In terms of Cq value, the highest expression was found in the PI 371795 female flower and the lowest was in the control sample. Cq is the basic principle of qPCR and is important information in producing accurate data [18]. The value of Cq is obtained from the number of cycles in the qPCR process that intersect the threshold line, which is indicated by a significant increase in the fluorescence signal [19].

The results showed that the expression of the CmACS-7 gene in female or hermaphrodite flowers was greater than that of male flowers (Table 3). Sexual expression in melons is regulated by genes responsible for ethylene biosynthesis [20]. The andromonoecious gene is responsible for the formation of 1-aminoacyclopropene-1-carboxylic acid synthase (ACS) which refers to the CmACS-7 gene homologous to the ACS-7 gene in Arabidopsis which contains genes that express hormones that play a role in determining sex in melons [7]. The result of research by Martin [20] showed that the CmW1P1 gene expression played a role in the transition from male to female flowers in melons. The CmW1P1 gene expression is responsible for inhibiting pistil formation and indirectly suppresses the CmACS-7 gene expression for stamens development in melons so that the interaction between these two genes produces sexual types in melon plants [21].

About two-thirds of melon cultivars in the world are Andromonoecious types and the rest are sexually monoecious types [2,8]. Plants of the monoecious sexual type provide control in pollination, seed production, and fruit quality. Plants with monoecious sexual types are at low risk of being infected by pathogens and produce better quality fruit [22,23]. Although currently many cultivars of melon are sexually andromonoecious types, cultivars with the sexually monoecious type continue to be developed because of their agronomic advantages [24]. According to Loy [25], Melon fruit that develops from female flowers has many advantages, including larger size, thicker flesh, and resistance to disease. These data provide interesting insights into the role of sexual types of Cucurbitaceae plant and also reveal some of the genetic basis for determining sex with hormonal influence.

4. Appendices
Based on the results of the study, it could be concluded that by means of the relative quantitative qPCR analysis, the expression of the CmACS-7 gene in female or hermaphrodite flowers was greater than that of male flowers. In male flowers with andromonoecious sexual types, the expression of the CmACS-7 gene was greater than that of monoecious male flowers. The CmACS-7 gene was expressed in all flowers on various sexual types of melon plants with varying levels of expression.
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